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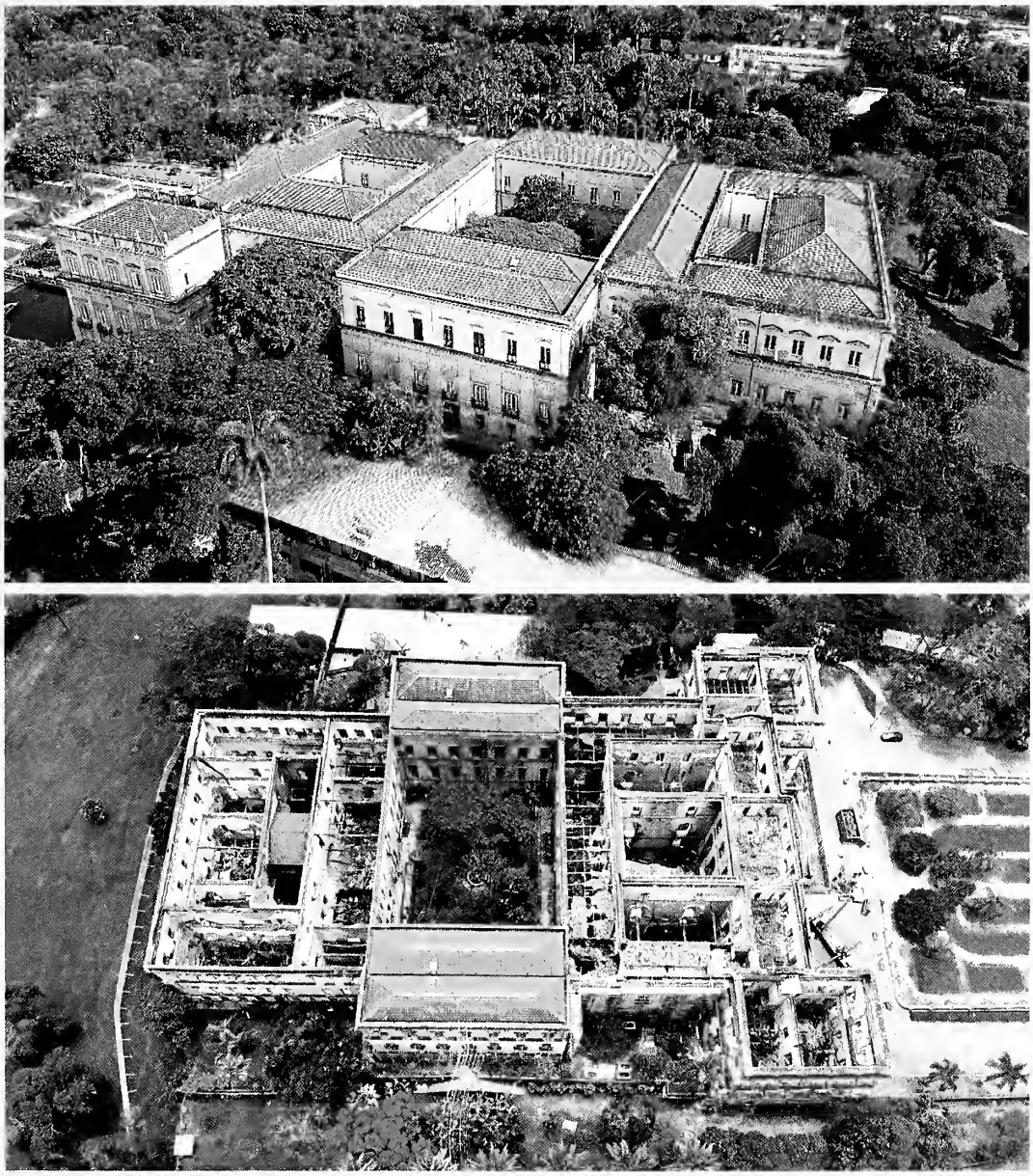

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Cover photos: Museu Nacional, Rio de Janeiro before (above) and after (below) the fire of September 2, 2018, which destroyed the Arachnology collection along with the other museum holdings. The ArachnoLab is at the lower right in the lower photo. See *Immolation of Museu Nacional, Rio de Janeiro – unforgettable fire and irreplaceable loss*, pp. 556-558; aerial photos by José Roberto da Silva.

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Bad tenants: female sheet-web spiders, *Cambridgea foliata* (Araneae: Desidae), lose feeding opportunities when cohabiting with males

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Abstract. In web-building spiders, females are often too widely distributed across the landscape for males to monopolize more than one mate. Consequently, males seek females one at a time and may cohabit with females in their webs. Pre-copulatory cohabitation is most common in araneomorphs, which suggests that the first male to mate with a female will have a greater share of paternity than any subsequent mates (first sperm precedence). However, pairs of adult New Zealand sheet-web spiders (*Cambridgea foliata* (L. Koch, 1872); Desidae) cohabit for longer than required to achieve copulation. This is counter-intuitive as it suggests that males defend females they have already copulated with, in lieu of seeking additional mating opportunities. To investigate the costs and benefits of extended cohabitation on male and female *C. foliata*, we conducted surveys of webs of solitary and paired males and females. We found that solitary spiders of both sexes consistently position themselves in the center of their webs but that when in pairs, females are displaced from the webs by males and will frequently leave the web altogether. Males in pairs would respond to vibrations simulating prey, while females would not respond. This strongly suggests that extended cohabitation should be costly for females. By contrast, for males, cohabitation is a valuable foraging strategy which, combined with the advantages of mate-guarding, may compensate for any lost mating opportunities due to foregoing searching for further mates.

Keywords: Mate-guarding, sperm competition, behavior, spider, New Zealand

Males regularly compete to ensure both mating success and the likelihood that their sperm will be used by females to fertilize their eggs (Parker 1970). This competition can manifest in a variety of ways, including defense of mates or resources, and scramble competition. In the former, males compete through agonistic contests or displays to defend spatially clustered females or resources important to females (Emlen & Oring 1977; Thornhill & Alcock 1983). In the latter, if females are widely distributed or are only available for a limited time, defense is not economical so males scramble to mate with as many females as possible. This seems to be a relatively common mating system among spiders (e.g., desert spiders, *Stegodyphus lineatus* (Latreille, 1817), (Berger-Tal & Lubin 2011); redback spiders, *Latrodectus hasselti* Thorell, 1870 (Kasumovic & Andrade 2009); bowl and doily spiders, *Frontinella pyramitela* (Walckenaer, 1841) (Austad 1984); golden orb spiders, *Nephila plumipes* (Latreille, 1804) (Kasumovic et al. 2007)).

However, while it is advantageous for male spiders to mate multiply over their life time, brief periods of cohabitation, a form of mate-guarding, are not uncommon. In spiders, mate-guarding males will share nesting chambers (e.g., jumping spider *Bavia aericeps* Simon, 1877) or webs (e.g., *Inola subtilis* Davies, 1982), or remain on secondary structures of the females' webs (e.g., *Araneus anatipes* (Keyserling, 1887) reviewed in Jackson 1986). Male spiders predominantly cohabit with subadult females with records for 161 species in which adult male araneomorphs cohabit with conspecific juvenile females (Jackson 1986). In these cases, males often mature earlier than females, find a female one or two molts from maturity and remain with her until she matures.

This predominance of males cohabiting with subadult females is logical, provided that the first male to mate with a female receives some advantage over subsequent males. If that male alone mates with the female and she oviposits shortly after or becomes unreceptive, then he will secure 100% paternity for her clutch. However, while cohabitation with subadults is more common, post-copulatory cohabitation with adults does occur, even at the cost of additional mating opportunities (Parker 1974). Alcock (1994) identifies several alternative hypotheses to explain the evolution of prolonged male-female associations following copulation. Mate-guarding may prevent females from accepting additional copulations (Thornhill 1984) or favoring the ejaculates of subsequent males (Eberhard 1991). In species that exhibit "last sperm precedence" in which subsequent mates receive a greater share of paternity, it is valuable for males to guard their mates (Austad 1984) in some cases until oviposition (e.g., cellar spiders, *Pholcus phalangioides* (Fuesslin, 1775), Schaefer & Uhl 2003) or until females enter a refractory period during which they are unreceptive to further mating (e.g., marbled cellar spiders, *Holocnemus pluchei* (Scopoli, 1763), Calbacho-Rosa et al. 2010).

Males may also associate for longer in order to mate multiply and increase their share of paternity (Simmons 2001). For example, male leaf-curling spiders (*Phonognatha graeffei* (Keyserling, 1865)) improve fertilization success through multiple, prolonged copulations and for this reason cohabit with adult females for several days (Fahey & Elgar 1997). While only a short period of pre-copulatory cohabitation may be needed to ensure a single copulation, longer periods of cohabitation may maximize fertilization success for the male.

Nevertheless, cohabitation should be costly to males as they forgo seeking additional mates (Birkhead & Moller 1992; Fryer et al 1999; Harts & Kokko 2013). However, these "costs" assume not only that there are a large number of receptive females available elsewhere but also that, if they should locate additional mates, males would not meet any resistance from other guarding males (Harts & Kokko 2013). Furthermore, remaining in a female's web may provide additional benefits not directly related to reproduction, such as safety. Austad (1984) remarks that post-copulatory mate-

guarding should only be advantageous if moving between webs, and finding a newly molted female is risky. This risk of travelling has been demonstrated in several spider species in which less than a quarter of males observed in a female's web are likely to be found again in the web of another female (e.g., golden orb web spider, *Nephila clavipes* (Linnaeus, 1767), Christenson & Goist 1979; bowl and doily spider, *Frontinella pyramitela*, Austad 1984; redback spider *Latrodectus hasselti*, Andrade 2003). Predation by other invertebrates is likely to be greatest source of mortality (Andrade 2003). Consequently, it can be advantageous for males to maximize their reproductive output with a resident female before rather than risk searching for another.

Cohabitation may also provide males with greater feeding opportunities. Suter & Walberer (1989) found that male bowl and doily spiders (*Frontinella pyramitela*) feed on prey caught in female's webs and suggest that this could be why cohabitation can go on for several days, far longer than is necessary for only courtship and copulation. They suggest that this may compel the female to eventually expel her tenant. Furthermore, Erez et al. (2005) demonstrated clear benefits of cohabitation for males at the expense of resident female desert spiders, *Stegodyphus lineatus*. When they provided food to webs containing cohabiting pairs, the males gained in condition while females did not. In species that depend on capture webs to feed, foraging in a resident female's web is an ideal way for a male to prepare for searching for his next mate (Austad 1983). Apart from these studies, there are no other examples that we are aware of which test whether males gain feeding benefits while cohabiting on female webs.

Cambridgea foliata (L. Koch, 1872) (Desidae) are arboreal spiders distributed throughout the North Island of New Zealand. They are nocturnal and build three-dimensional sheet-webs in native forest which males and females can be seen sharing. The webs consist of a non-sticky, thick horizontal mainsheet anchored from below, with many knock-down threads above the mainsheet. The spiders run along the underside of the mainsheet. The rear of the web tapers into a silken tunnel or "retreat" which may extend under the bark of trees or into vacated burrows; the spider resides in the retreat during the day. During the summer, males mature in their natal webs before leaving to wander in search of female webs. Once a male finds a female's web, he may cohabit with her. McCambridge (2017) found that *C. foliata* will cohabit for between one day to about 14 days, with a small number of males cohabiting with subadult females for more than 20 days. Based on the author's data and our own observations, we expect that one to two days is the most common duration for cohabitation. During this time, the cohabiting male defends the female's web by engaging in ritualized fights with intruding males (pers. obs). When males fight in a female's web, we have often observed the resident female departing the mainsheet and sitting either on one of the anchoring threads or off the web entirely. This suggests that one male's presence in females' webs, or a succession of cohabiting males, may entail some foraging cost to females. However, we have not observed any instances of sexual cannibalism or fighting between males and females, which are similar in size.

Courtship and mating behaviors have not been observed in this species. However, as most spiders take only a few minutes to copulate (Fahey & Elgar 1997), it is reasonable, barring the use of mating plugs or genital mutilation (reviewed in Huber 2005), to expect that cohabiting pairs have the opportunity to mate more than once. Males often cohabit with juvenile females— which is consistent with the predictions of Austad (1984) for cases of first sperm precedence—but males have also been observed cohabiting with adults. As *C. foliata* are entelegyne spiders with "conduit" or "one-way" type copulatory ducts, we may expect them to exhibit first sperm precedence. However invertebrate copulatory and fertilization duct morphology is highly variable, which can generate significant variation in the strength of first or last sperm precedence (Uhl 2000). For this reason, we do not assume whether this species exhibits first or last sperm precedence.

Given that male *C. foliata* cohabit with females for longer than required for a single copulation, in the present study we aim to assess the impact of males cohabiting with females by (1) comparing positions of male and female spiders on their webs when alone and when cohabiting, and (2) comparing responses of males and females to simulated prey. If we assume that the probability of prey landing is approximately equal at all points on the mainsheet, then the center of the mainsheet (which we call the "hub") should be the optimal location for the spider to sit, as it is approximately equidistant to all edges of the web and to the retreat where these spiders hide if disturbed. For this reason, we predict males and females will most commonly position themselves in the hub when alone. When in pairs, we expect that both sexes will spend less time in the hub compared to when alone but that males will spend more time in the hub than females, as the mainsheet serves as the arena on which males fight each other. Due to males and females sharing the web when in pairs, we expect that both will be less likely to approach a simulated prey item compared to when alone. Another objective is to describe courtship interactions on females' webs, which have not been described before and may be useful for understanding mate-guarding in this species.

METHODS

Courtship observations.—In addition to making observations of male-female interactions in the field, we collected juvenile *C. foliata* for a lab population which included females with a known reproductive history. Each spider was housed in a $30 \times 30 \times 60.1$ cm mesh enclosure. A wooden retreat was attached to the upper rear corner of the enclosure. We modelled these on wētā enclosures devised by Bowie et al. (2006). They consist of a ($45 \times 45 \times 150$ mm) block of untreated wood with a furrow (18×18 mm) cut two-thirds the length of the block, which was placed against the mesh wall. A black polyethylene flap was attached to the outside of the cage, covering the retreat. We fed the spiders every two days on a mixture of meal worms (*Tenebrio moliter*), blow flies (*Lucilia sericata*) and locusts (*Locusta migratoria*). Spiders were misted with water three times a day.

By the time females reached maturation their webs were dense enough to support multiple spiders. To observe courtship interactions, we introduced a random unmated

male to a random female's web and recorded subsequent behaviors for up to 3 hours.

Cohabitation surveying.—We surveyed *C. foliata* webs at night in Matuku Forest and Bird reserve in West Auckland (36° 51′ 48.3″S 174° 28′ 47.7″E). We observed webs on either side of the walking track across the 2015/2016 and 2016/2017 summer seasons. We could ensure that we did not observe the same individuals across the separate seasons as in 2015/2016 the spiders were subsequently collected for other research. Meanwhile in the 2016/2017 season, we ensured that we did not repeat observations of the same spiders by not visiting the same trees. We do not have data on the reproductive history of observed males and females.

When we found an adult spider in a web, we made a note of sex, location in the web and whether the spider was solitary or in a pair. The "locations" we included were: "retreat," meaning at the opening of the retreat or on the mainsheet immediately outside the retreat; "hub," referring to the approximate center of the mainsheet; "web," referring to all remaining parts of the mainsheet; and "off," indicating that the spider was either on the knock-down threads, on the guying threads or just off the mainsheet on the substrate within 10 cm of the web.

To examine whether spiders changed how they responded to a prey stimulus when in a pair compared to when alone, we provided an artificial stimulus simulating prey movement in the web. All surveys were conducted between 1 and 3 hours after sunset. In order to standardize the stimuli, we used a Wittner tuning fork with a pitch A440 (440 Hz). We had previously observed that *C. foliata* females consistently approach a vibrating tuning fork when it is touched to the mainsheet of the web, and will attempt to bite it before realizing that it is not a prey item. In the current study, we would strike the tuning fork and touch the tip lightly approximately 20 cm from spider's location. This controlled for differences in web size, which varied considerably (mean area = 3169.48 cm^2, SD = 1220.63).

We recorded whether the spider(s) approached, moved away from or did not respond to the stimulus. As most individuals either responded within 5 seconds of the stimulus being applied or did not respond for more than 30s or with additional applications of the stimulus, we did not record latency.

We surveyed the webs of 62 solitary females, 34 solitary males and 47 adult pairs. We collected response data for 35 solitary females, 22 solitary males and 23 pairs. When analyzing spider locations and responses, we excluded 11/62 of the webs with solitary females, 3/34 webs with solitary males and 5/47 webs with pairs because spiders were feeding. We kept these data separate to our other location data as a prey item falling into the web would draw one or both spiders away from their original location and therefore would not be representative of the "preferred" location.

Statistics.—We conducted a χ^2 test for homogeneity of variance on contingency tables for female locations depending on whether they were alone or in a pair. When tabulating responses to the stimulus, our expected count values for our contingency tables violated the assumptions for a χ^2 test of homogeneity. In the vast majority of responses, the spider either approached or did not respond; very few moved away from the stimulus. Thus, we pooled negative responses and no response together. The resulting contingency table for whether females approached the stimulus (with binary responses of yes or no) did not violate the assumptions for a χ^2 test.

Figure 1.—Male and female *Cambridgea foliata* in copulatory posture.

We calculated odds ratios for the probability that females would take up certain positions when in pairs compared to when alone, and for the probability that they would approach a stimulus as opposed to move away or not respond. We conducted these same tests for males. All analyses were conducted in R version 3.2.0. (R core team 2015).

RESULTS

Courtship.—We observed 10 instances of courtship by the male. Of these observations, 6 males entered a copulatory posture. When males first enter webs occupied by females, they may shake the web using their whole body. The female orients towards him and, soon after, approaches him. She touches him with her forelegs and then may retreat a short distance in the direction of the retreat. The male may continue to interact with the female or move past her and enter the retreat. The interactions we observed lasted for an average of 93.8s (SD = 168.3s). In one case, a male courted a female for 615s.

Often at the entrance of the retreat, the male shakes the web and dorso-ventrally flexes his abdomen. If the female approaches, he will drum on the web with his first and second pairs of legs. The female may angle her cephalothorax away from the web allowing the male to approach and place one palp over her epigyne (Fig. 1). The pairs which we observed remained in this copulatory posture for 382.8s (SD = 212.5). While the male's palp was placed over her epigyne at all times, the male's palp was never charged with sperm, meaning that sperm transfer could not have been occurring. Males also only ever used one palp. When the male moved out of this posture, he would either remain on the web a short distance from the female or return to the retreat.

Cohabitation.—We observed males cohabiting with both adult and subadult females and, in one case, we found an adult male sharing a web with a subadult male. We never observed

Table 1.—Positions of female *C. foliata* on webs.

Location	Solitary	Pair	Total
Hub	28	8	36
Retreat	4	13	17
Web	19	5	24
Off	0	16	16
Total	51	42	93

Table 2.—Positions of male *C. foliata* on webs

Location	Solitary	Pair	Total
Hub	16	20	36
Retreat	5	12	17
Web	10	10	20
Off	0	0	0
Total	31	42	73

copulation. When surveying for solitary males, we sometimes found individuals on webs produced by heterospecifics (e.g., *Badumna* sp. Thorell, 1890).

In addition to sharing webs during the night, males and females can frequently be found sharing retreats during the day. As adult males wander, we expect that these webs and retreats belong to the female. We observed that in the morning when the pair return to the retreat, the male will usually enter the retreat first with the female following (pers. obs.). Similarly, if the pair are disturbed during the night, the male will enter the retreat first regardless of whether he or the female is closer to it.

Beyond occasional observations from other research of marked males appearing in different females' webs over sequential nights, we do not have data to show how many females a *C. foliata* male may encounter in his lifetime. However, McCambridge (2017, pers. comm.) marked and recaptured 3/26 adult male *Cambridgea plagiata* Forster & Wilton, 1973 after 29, 43 and 49 days. While we cannot extrapolate these findings to the survival rate of males in general, these males should have encountered at least two females' webs.

Preference for location.—When alone, males and females demonstrated very similar preferences for different locations in the web. Solitary females were most often found in the hub of the web or near the retreat (Tables 1, 2). Similarly, solitary males were most often found in the hub or at the retreat (Fig. 2). Indeed, the hub was the most frequented location for spiders alone in the web, regardless of sex. The second most common location for the spiders was off center from the hub but more than 10cm from the edge of the web. When we observed spiders feeding (both in pairs and alone), they were similarly found in or near the hub.

Male distribution did not change significantly when in a pair compared to when alone ($\chi^2 = 1.708$, df = 2, $P = 0.426$). Males in pairs were still most often found at the hub, although they were more likely to be found at the retreat compared to solitary males (odds ratio, OR = 1.77). By contrast, we found that females in pairs placed themselves differently on the web compared to lone females ($\chi^2 = 40.109$, df = 2, $P < 0.0001$). Females in pairs were less likely to be in the hub or elsewhere on the mainsheet (OR = 0.346, 0.320 respectively). They were 3.95 times more likely to be at the retreat. However, the largest share of females in pairs (38.1%) were found off the web entirely. They were sometimes found on the knock down threads, but most frequently on the guying threads. They were never seen off the web when alone, making it impossible to calculate a meaningful odds ratio.

Responses to stimulus.—When alone, the majority of males and females approached a vibratory stimulus (Fig. 3, Tables 3, 4). However, while solitary females almost always approached, males sometimes moved away or did not respond. For cohabiting females, we found significant evidence that the presence of a male in the web affected the likelihood of approaching a stimulus ($\chi^2 = 42.437$, df = 1, $P = <0.0001$). Females in pairs were 31.96 times more likely not to respond or to move away from the vibratory stimulus, with 20/23 females in pairs not responding to the stimulus at all compared to only 1/35 of solitary females. Of the 20 paired females which did not respond, 10/20 were on the mainsheet (retreat, mainsheet or hub) while 10/20 were off the web (guying threads, knockdown threads, off entirely). This suggests that

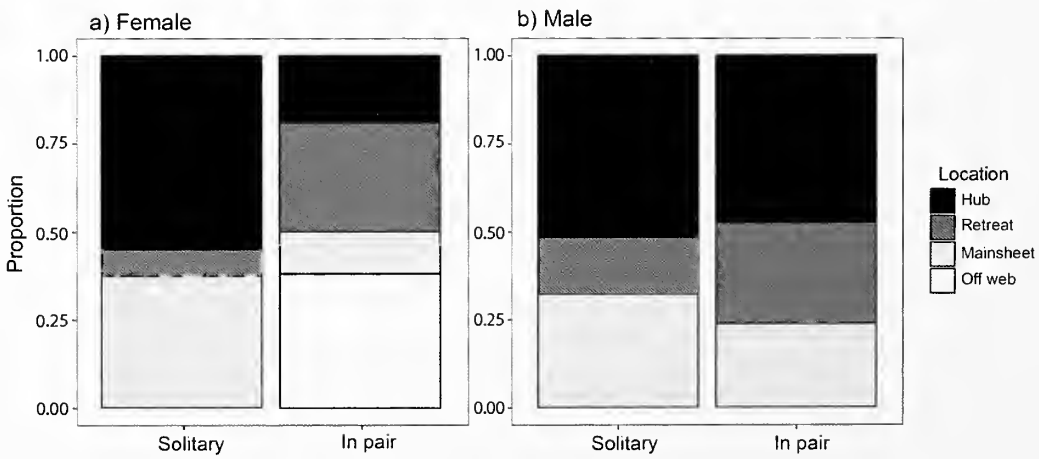

Figure 2.—Proportion of webs in which (a) females and (b) males were found in the hub, retreat, mainsheet (web) or off the web depending on whether the spider was alone or in a pair.

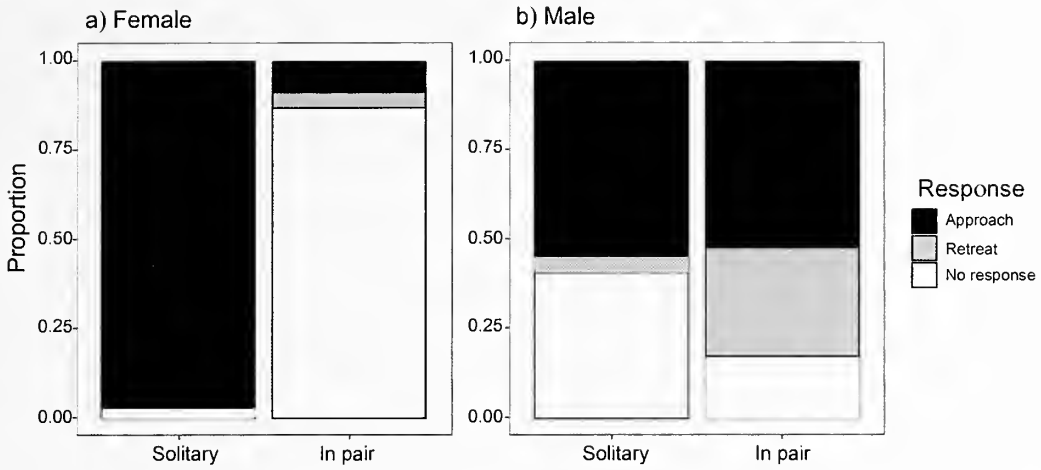

Figure 3.—Proportion of webs in which (a) females and (b) males approached, moved away from or did not respond to the artificial prey stimulus depending on whether they were alone or in a pair.

even females who are still in contact with the main web (and able to feel the vibration) did not respond to the stimulus.

There was also no evidence that males were more likely to approach a stimulus when sharing a web compared to when alone (OR = 0.957; $\chi^2 = 0$, df = 1, $P = 1$).

Feeding behavior.—We observed 19 instances of spiders feeding. In total, spiders were most often observed feeding in the hub closely followed by elsewhere on the mainsheet (Table 5). We observed one solitary female feeding on the knockdown threads. Within the cohabiting pairs we observed, we only saw males feeding.

DISCUSSION

While *C. foliata* of both sexes most frequently placed themselves in the hub of their web and approached prey when alone, male and female behavior diverged significantly when cohabiting. When in a pair, males were still found most frequently in the hub and still approached a stimulus mimicking prey. By contrast, females were often off the web entirely and almost never approached our stimulus. Cumulatively, this suggests that cohabitation is disadvantageous for females but advantageous for males.

The *C. foliata* mating system is best described as "prolonged searching polygyny" (Herberstein et al. 2017). It is advantageous for males to find unmated females rapidly and males depart webs in search of other webs. However, at each female's web, males may invest significant time into cohabiting. The hub of the web is equidistant from all edges of the sheet and the retreat, suggesting that it is probably the location which minimizes distance to both prey and to safety. It is unsurprising, then, that we predominantly found solitary females and males in the hub of the web.

However, while males in pairs largely continued to place themselves in the hub when cohabiting, a large proportion of females in pairs were found off the mainsheet entirely. In this way, it is unsurprising that so few females responded to vibrational signals given that they were less likely to detect the vibrations or be in a position to rapidly take prey. However, even females that remained on the mainsheet in the presence of a male were less likely to approach the stimulus compared to when alone. In this way, females that cohabit with males for an extended period or with a series of males almost certainly forgo most, if not all, foraging opportunities. Given that when we observed pairs, we only saw males feeding, it is reasonable to expect that if cohabitation were prolonged, male condition would improve at the expense of female condition, as has been found in desert spiders (*Stegodyphus lineatus*; Erez et al. 2005).

An extended period of cohabitation would also be particularly advantageous for males if travelling between females' webs were risky (Austad 1984). In other spider species, there is a high mortality rate for males travelling in search of females, most likely as a result of predation (e.g., Christenson & Goist 1979; Andrade 2003; Berger-Tal & Lubin 2011). It would be worth investigating whether this is also true for *C. foliata*.

At least, the value of webs as a source of food is apparent in how males take advantage of abandoned webs which, naturally, provide no reproductive opportunities. We sometimes observed males in webs previously inhabited by females who had disappeared after storms. We even observed one male living on a web built by a house spider (*Badumna* sp.) suggesting that males can be truly opportunistic about finding refuges while roaming. We expect that the majority of solitary males we found were inhabiting webs built by spiders other

Table 3.—Female responses to simulated prey.

Response	Solitary	Pair	Total
Approach	34	2	36
Move away	0	1	1
No response	1	20	21
	35	23	58

Table 4.—Male responses to simulated prey.

Response	Solitary	Pair	Total
Approach	12	12	24
Move away	1	7	8
No response	9	4	13
	22	23	45

Table 5.—Number of spiders observed feeding in different web localities.

	Solitary		Paired		
Location	Male	Female	Male	Female	Total
Web	2	4	1	0	7
Hub	1	5	2	0	8
Retreat	0	1	2	0	3
Off	0	1	0	0	1

than themselves, given that the adult males we kept in the lab did not build webs. This cumulatively suggests that the most important advantages of cohabiting may be directed towards male survival.

Our primary limitation to fully understanding the benefits of cohabiting is that we do not yet know how often cohabiting pairs copulate. While we observed males placing only one palp over the female's epigyne during courtship, our males never charged their palps with sperm prior to this and we never observed copulation in the cohabiting pairs we found in the field. Based on our behavioral observations, we expect that courtship and copulation should occur at least once more, after the male has charged his palps with sperm, if not on the web during the night then in the retreat during the day. If a single copulation does not ensure paternity then guarding is essential to ensure male fertilization success which would make sperm competition the primary benefit of guarding rather than safety and/or foraging opportunities.

Furthermore, as we do not know the sperm precedence patterns for this species, it is difficult to estimate to what extent cohabitation reduces sperm competition. It is possible that if females re-mate, subsequent males may gain a proportion of paternity, making mate-guarding an adaptive strategy. *Caubridge foliata* are entelegyne spiders, generally thought to have first sperm priority, which should lessen the value of post-copulatory cohabitation (Austad 1984). However, it has been demonstrated in other entelegyne spiders that the second male may gain between a 5% share of paternity (*Frontinella pyramitela*; Austad 1982) to near parity (46% in *Nephila plumipes*; Schneider & Elgar 2001) or even the majority (66% in *Nephila edulis*; Schneider et al. 2000). Regardless, even if re-mating by the female merely reduces the first male's share of paternity, a male will benefit from mate-guarding, particularly if there are additional benefits to remaining with the same mate.

Extended cohabitation with adult females is a relatively uncommon form of mate- guarding in spiders, and cohabitation beyond the time required for copulation seems particularly uncommon. Furthermore, in New Zealand sheet-web spiders, cohabitation seems particularly costly to the female, who frequently leaves the mainsheet of her web when in pairs and most likely loses foraging opportunities as a consequence. By contrast, cohabitation has the potential to be highly beneficial for males. First, it helps males to control mating by allowing multiple copulations and preventing re-mating by the female. Second, it provides males with feeding opportunities and likely keeps males safe from heterospecific predators which they may meet when searching. Determining when copulation occurs, how frequently it occurs and how sperm competition manifests when females mate multiply is critical to understanding the value of cohabitation in comparison to searching for additional mating opportunities in this species. However, male and female behavior on webs when in pairs compared to when alone provides an explanation as to why males frequently cohabit following copulation rather than continue searching for additional mates.

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Mating and egg-laying behavior of *Hasarius adansoni* (Araneae: Salticidae) and the influence of sexual selection

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Abstract. Jumping spiders perform multi-modal displays during courtship and this has been used to study sexual selection and mate choice. However, studies have focused on only a few groups of spiders. Here we describe for the first time the breeding behavior of the tropical jumping spider *Hasarius adansoni* (Audouin, 1826). Animals were collected in the field and reared in the laboratory until adulthood. We took male body measurements, paired couples in mating trials, and then collected subsequent clutches. We confirmed the presence of a multi-modal display with visual and vibratory signals (tremulations) by the males. Females responded with their own tremulations and occasionally a receptive posture. Otherwise, they avoid mating by attacking or running away from the male. No measured male morphological attributes were important for male mating success and future studies should focus on other morphological measurements to understand how the mate choice process functions in this species. Multiple matings were common and females laid numerous clutches while enclosed in silk cocoons. Number of young per clutch decreased over the course of laying bouts.

Keywords: Jumping spiders, multi-modal display, mate choice

Salticid spiders have excellent vision (Levi & Levi 1990; Hill & Richman 2009) and most of their behaviors are visually guided (Richman & Jackson 1992). Courting behavior is no exception, and males from this family are known for performing extravagant visual and vibratory displays to attract females (e.g., Jackson & Macnab 1989a, b; Hill & Richman 2009; Girard et al. 2011). In many species, females respond with their own display behavior (Levi & Levi 1990; Cross et al. 2007). Recent work has shown that jumping spiders also produce vibratory signals, and these are often complex and coordinated with visual displays (Foelix 2011; Elias et al. 2012). For these reasons, salticids are important models for studies of the evolution of communication, including hypotheses about signal elaboration, multi-modal signals, and signal function across diverse habitats. Moreover, male sexual displays in Salticidae are important in speciation and can be key characters for taxonomic classification (Richman 1982; Masta & Maddison 2002). Richman (1982) presented a comprehensive description of the displays of species across genera, information that is critical for salticid systematics. However, as is the case for many spider families, behavioral data are available for relatively few species, and there are entire genera with little or no information available. In the case of salticids, behavioral studies are concentrated in groups of the genera *Habronattus* F.O. Pickard-Cambridge, 1901 and *Phidippus* C.L. Koch, 1846, focusing mostly on breeding behavior (e.g., Sivalinghem et al. 2010; Elias et al. 2012; but see Clark & Morjan 2001 and Lim et al. 2007 for examples in other genera). This hampers studies of the evolutionary history of this group and precludes comparative analyses of signal evolution.

Here, we examine the breeding behavior and sexual signals of *Hasarius adansoni* (Audouin, 1826), a salticid that is common in urban environments throughout the tropics (Levi & Levi 1990). Despite its widespread distribution, (Levi & Levi 1990; Zabka & Pollard 2002), this species has been the subject of only one behavioral study to date. Cloudsley-Thompson (1949) provided some descriptive notes about *H. adansoni* sexual behavior, including display, copulation and egg-laying behaviors. However, this was based on a very small sample size, largely anecdotal observations, and since no viable eggs were produced it is unclear whether matings were successful. However, Cloudsley-Thompson's description suggests *H. adansoni* males produce visual signals, and this is also suggested by their sexually dimorphic coloration; while females are cryptic brown, males are black with conspicuous white patches on their palps (Levi & Levi 1990; Fig. 1). Thus, the objective of this study is to describe this species' display, copulation and egg-laying behaviors. Specimens of both sexes of *H. adansoni* are deposited in the arachnid collection of the Universidade de Brasília (UnB), Laboratório de Aracnídeos, collection number 4304.

METHODS

Rearing.—A total of 94 animals were used in mating experiments. We captured *H. adansoni* juveniles before their last instar on urban walls and buildings around the city of Brasília, Brazil (15°45′47.4″ S, 47°52′14.3″ W) and brought them to the Laboratório de Comportamento Animal in Universidade de Brasília (UnB) where mating trials were conducted. Vibratory signals produced by one pair were recorded at the University of Toronto Scarborough (43°47′1.47″ N, 79°11′15.66″ W). We could not repeat vibratory analysis with other pairs, since most of those transferred to Canada were part of other experiments. No permits were needed to transport the live spiders to Canada. All animals were kept in cylindrical glass containers measuring 9 cm X 4.5 cm in natural photoperiod and room temperature. A piece of wet cotton was kept inside each container to

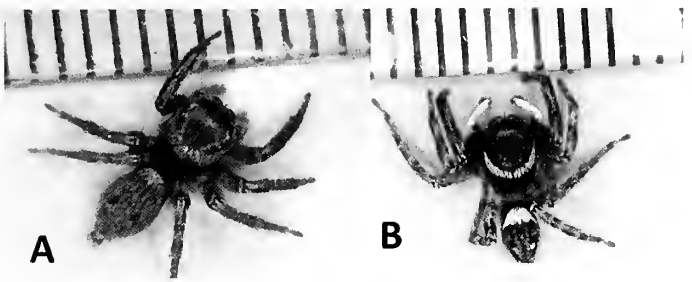

Figure 1.—Sexual dimorphism in *Hasarius adansoni*. A. Female; B. Male.

maintain moisture. Spiders brought to Canada were maintained in similar conditions. Animals were fed every four to seven days. In each feeding episode, individuals were given 10 to 15 *Drosophila* spp. and one young *Gryllus* cricket. We also fed the spiders on the day before conducting the mating experiment described below.

Vibratory signals.—Since substrate-borne vibratory signals are common in salticid spiders (Foelix 2011), we used one pair of spiders to determine whether such signals occur for this species. The pair was placed together in a circular mating arena (11 cm diameter) on a turntable covered in stretched nylon and with all sides composed of a clear plastic wall. This type of arena has been previously shown to transmit salticid courtship signals (Elias et al. 2003). Laser Doppler vibrometry (LDV, PDV100 portable laser vibrometer, Polytec, Tustin CA, USA) was used to detect the occurrence of substrate vibrations during the pair's interactions. Vibratory signals were recorded, along with detailed videos with sound available that made it possible to detect any movements causing vibrations by the animals. Three small pieces of lightweight reflective tape (~1 mm) were placed near the center of the nylon-covered turntable and used as measurement points for the laser. Laser output was fed through a speaker to allow real-time audio monitoring of vibratory signals. Simultaneously, the pair was filmed using a digital high-speed camera (500 frames s^{-1}; PCI 1000; RedLake Motionscope, San Diego, CA, USA) while the spiders were illuminated with a Minifill light, manufactured by Frezzi. For this exploratory analysis, we monitored the high-speed video while listening to the LDV output to determine candidate body movements that might generate vibratory signals (e.g., Elias et al. 2012).

Mating trials.—A total of 47 mating trials were recorded on digital video during the experiments (excluding the trial on vibratory signals), and males and females were used only once. For these trials, the mating arena consisted of a square acrylic container (13 cm x 13 cm X 4 cm) with two opaque dividers that allowed two spiders to be held simultaneously without visual contact. The container also had niches in the four corners where spiders could avoid each other. For every trial, one adult male and one adult female were held inside the arena but kept apart by the opaque dividers, which were simultaneously opened to start the experiment after a 1-h acclimatization period (Fig. 2). Age, measured as days since last molt, were 50.8 (±70.2) days for males and 51.2 (±73.81) days for females. Each pair was videotaped for 3h (Kodak Zx1 Pocket Video Camera), from the upper side of the arena, and all

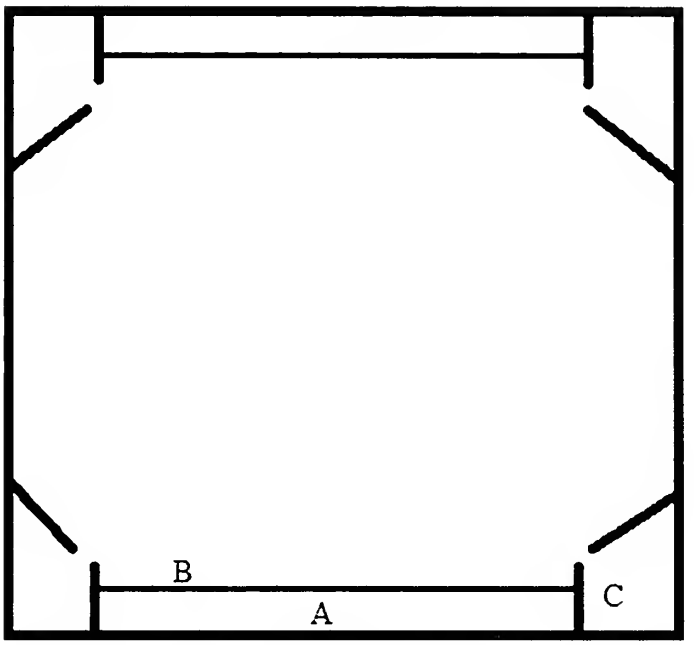

Figure 2.—Upper view of the mating arena. A. Compartment to hold spiders; B. Opaque doors that can be opened; C. Refuge. Arena dimensions: 13 × 13 × 4 cm.

experiments were conducted under a natural light-simulating lamp (Arcadia Bird lamp. Model FB 36).

The videos were then analyzed to develop an ethogram of the three stages of breeding for both males and females: (i) pre-copulation display and response; (ii) copulation behavior; and (iii) post-copulation behavior (i.e., egg-laying behavior). Below we describe the behavioral repertoires, time spent in each of these phases and number of eggs and young produced by *H. adansoni*.

Measurements.—Before every trial, males were weighed to the nearest 0.001g. After each trial males were measured and then sacrificed, and their palps and front legs (used in the courtship, see below) removed for measurement. Every measurement was done by photographing the animals with a stereomicroscope, keeping a ruler in the image for scale. Images were then entered in ImageJ for measurement. Carapace width was used as a measure of animal size. We also measured front leg length, white patch area and percentage of pedipalp covered with the white patch area. Areas were calculated drawing a polygon around palps and white patches and then extracting the area of the polygon. To summarize male morphology, cephalothorax width, leg length, male mass, white patch area and percentage of white patch cover were entered in a Principal Component Analysis (PCA). All morphological measurements were taken using pictures similar to those in Fig. 3. We assessed whether any morphological traits predicted mating success, using a regression analysis including the number of copulations as the response variable, along with male morphology (as predicted by the PCA) and male condition as predictive variables. Total duration of copulations was used as response variable in non-parametric regression analysis, since these data were highly overdispersed. Male condition was calculated as the residuals of the regression between male weight and male

Figure 3.—Morphological measurements taken from *Hasarius adansoni* individuals. A. Body size (carapace width; for males and females); B. Area of palp and area of white patch (males only); C. Length of leg I (males only).

cephalothorax width, as proposed by Jakob et al. (1996). Results are presented as mean ± standard deviation.

RESULTS

Mating trials.—We had nine trials in which animals did not see each other, and were considered unsuccessful and thus excluded from further analysis. Among our 38 successful trials (where animals saw each other), 23 (60.5%) resulted in copulations. Among those that did not result in copulations, only four were because males did not attempt copulation and one was because the female cannibalized the male. The other 10 were because females did not accept males (see description below).

When the male orients and moves towards the female, he typically spreads the first pair of legs and his palps (33/38 successful trials). Given the location of the white patches, this would reveal them to a female oriented towards him. From seeing a female and starting a display, males took a mean of 10.6 ±14 s, showing high variation in latency to court. The male then walks towards the female in a zig-zag fashion. Here, the female may respond in three ways: (i) facilitate palp insertion by curling her legs close to her abdomen and staying motionless; (ii) avoid palp insertion, by running away or (iii) avoid palp insertion, by attacking the male. If the first option happens (23/38 successful trials), the male can approach and mount the female, and she then exposes the side of her abdomen and this facilitates palp insertion. Latency to adopt receptive posture once the male is courting was 11.8 ±10.8 s, again showing high variation. Males courted females an average of 53.3 ± 30.86% of times they saw females. On the other hand, females rejected a mean of 45.5 ± 36.6% of males' attempts.

Palps are not inserted simultaneously, thus each insertion was counted as a separate copulation. Mean palp insertion duration was 22.96 ± 14.86 s. Pairs that copulated did so an average of 5.82 times (min = 1; max = 18). Although two separate insertions could happen in a row, multiple copulations were usually separated by a period of other behaviors, such as wandering around the arena, self-grooming, and many times, spiders lost visual contact with each other. Usually, males continued courting and mounting the female multiple times until she moved out of the receptive posture. Once this happened, females frequently adopted the second possible response to courtship (i.e., attacking or running away from the male). Cannibalism of the male by the female was extremely rare, and was observed only once in our 47 trials.

All the behaviors related to reproduction are represented in the ethogram in Table 1.

Vibratory signals.—We confirmed the presence of substrate-borne vibrations during courtship from both male and female. These appeared to be primarily tremulations, a type of substrate-borne vibration signal in which a part of the spider's body vibrates but does not touch the substrate. The energy of such vibrations, however, is transferred to the substrate by the spiders' legs and allows communication (Uhl & Elias 2011).

In this exploratory trial, when the male started moving towards the female, he used tremulation of the abdomen to create vibrations that were detected by the LDV and likely were also available to the female (Elias et al. 2004, 2005, 2006, 2012; Sivalinghem et al. 2010). Once in the receptive posture (i.e., legs curled but still touching the substrate), the female started her own abdominal tremulations as the male approached.

Reproduction.—An average of 36.25 ± 29.92 days after mating, females build a silk cocoon and stay enclosed for an average of 21.21 ± 12.1 days while laying eggs. Usually, after the female leaves the cocoon, the young molt for the first time and only then do they disperse. Among the females that mated, 69.5% ($n = 23$) laid viable eggs. Considering just the females that copulated, the number of copulations did not predict the likelihood of laying viable eggs (Binomial Model: $\beta = 0.054$; $P = 0.567$).

Mated females laid between zero and nine clutches (mean = 3.13) after mating. The number of young per clutch varied from zero (eggs failed to hatch) to 41. The number of young per clutch decreased over the laying bout for each female (Fig. 4), as shown by a mixed-model with Gaussian error distribution, entering the number of young as response variable, clutch as predictor and female identity as a random factor. Number of young correlated negatively with clutch number ($\beta = -1.87$; $P < 0.01$), but did not correlate with female size (Spearman's $\rho = 0.07$; $P = 0.6$, $n = 19$). Female condition predicted the number of young (Spearman's $\rho = 0.64$; $P = 0.002$, $n = 19$). However, such a relationship disappeared after the removal of one single outlier (Spearman's $\rho = 0.19$; $P = 0.44$, $n = 18$).

Morphology and mating success.—The first principal component of the PCA explained 63.9% of the total variance in the traits measured and was highly correlated with leg length, cephalothorax width, and mass; and moderately correlated to white patch area. The second principal component explained another 23.28% of the variance and was highly correlated to

Table 1.—Ethogram of mating behaviors of the jumping spider *Hasarius adansoni*.

Behaviors	Description
Male behaviors	
Leg spreading	Spreading the first and, sometimes, second pair of legs in the horizontal plane
Tremulation	Vibrating the abdomen, but not touching it on the substrate. Vibrations are of long duration (~0.5s) and high amplitude
Zig-zag approaching	Walking towards the female in a zig-zag fashion while performing leg spreading and tremulation
Palp insertion	Inserting the palp in the female epigynum. It happens right after zig-zag walking. Male is mounted on the female's dorsal side, facing her abdomen. Right palp is inserted in left epigynum or left palp is inserted in right epigynum
Female behaviors	
Receptive posture	Female curves all 8 legs towards the center of the body and stands motionless
Tremulation	Vibrating the abdomen, but not touching it on the substrate. It happens while performing receptive posture and vibrations are of short duration (~0.25s) and low amplitude
Abdomen turning	Performed when the male is executing palp insertion. It consists of a small torsion of the abdomen in the vertical plane that facilitates palp insertion by the male

percentage of white patch cover and also moderately correlated to white patch area (Table 2). This shows that the variance in white patch area is partly associated with both body size and percentage of cover. Thus, we used the first principal component as a measure of body size and white patch size and the raw values of percentage of white patch cover in subsequent regression models.

Among the females that copulated, number of copulations was not predicted by male size or percentage of white patch cover (Negative Binomial Model; PC1: $\beta = -0.29$, $P = 0.18$; white cover: $\beta = 1.44$; $P = 0.58$). Among these females, number of copulations also did not correlate with male condition (Negative Binomial Model; Condition: $\beta = 56.52$; $P = 0.43$).

The probability of copulation was not predicted by male size or percentage of patch cover (Binomial Model; PC1: $\beta = 0.47$, $P = 0.26$; Percentage of white cover: $\beta = -5.4$, $P = 0.43$). Furthermore, male condition and probability of copulation were not correlated (Binomial Model; Condition: $\beta = -82.55$, $P = 0.44$).

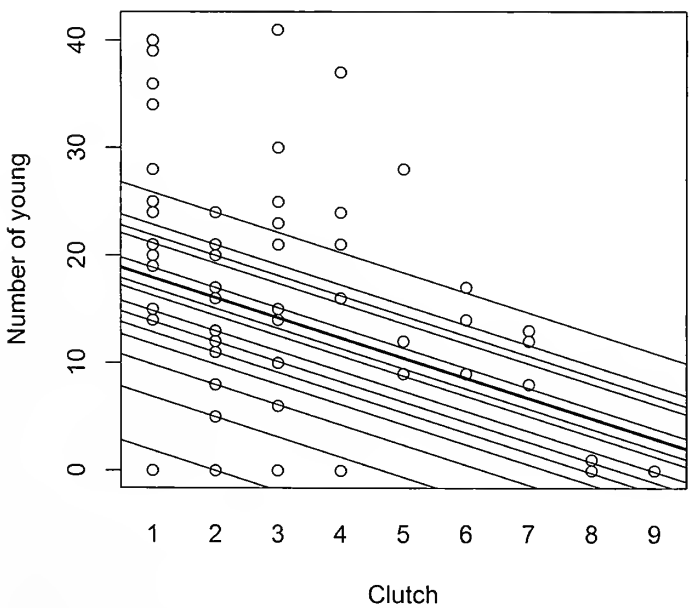

Figure 4.—Number of young and clutch number (i.e., 1st, 2nd, ...ninth egg sac) laid by female *Hasarius adansoni*. Each line represents a different female. Individual intercepts and slopes were extracted from a mixed-model with Gaussian errors, including number of young as the response variable, clutch number as predictor and female identity as a random factor.

Total copulation time did not correlate with any of the predictor variables (PC1: Spearman's $\rho = 0.15$, $P = 0.47$; Percentage of patch cover: Spearman's $\rho = -0.15$, $P = 0.45$; Condition: Spearman's $\rho = 0.11$, $P = 0.52$).

DISCUSSION

Jumping spiders produce relatively intricate displays (Richman & Jackson 1992) and our observations show complex, multimodal displays are also a feature of mating in *H. adansoni*, with males producing tremulations during approach, and females responding with their own tremulations in turn. Even though our sample size for vibratory signals is just one, we confirmed tremulation by both sexes and previous studies have found abdomen vibrations to be ubiquitous in the Salticidae (Uhl & Elias 2011), so we believe it is also common in *H. adansoni*. We found high levels of prolonged courtship by male *H. adansoni*, and clear receptivity postures among females. Multiple copulations were common within pairs that mated. Among mating females, the first clutch typically had the most offspring, and this number declined with subsequent clutches. Surprisingly, despite a high frequency of mate rejection (11/38 pairings), we could detect no relationships between male body size and condition, or the white patch on male palps and any of our measures of mating success or copulation frequency. Notwithstanding these results, it remains clear that this species may be useful to test hypotheses about breeding behavior and sexual selection, given the combination of visual and vibrational signals in the male displays, and the different behavioral and vibrational responses from females.

The features that compose the visual display in *H. adansoni* (i.e., leg spreading, zig-zag walking and palp spreading) have been observed in other salticid species (Richman 1982). Similarly, substrate-borne vibrations have also been observed during courtship in many Salticidae, although the type of vibrations and repertoire size vary substantially (Elias et al. 2003, 2005, 2010, 2012; Sivalinghem et al. 2010; Girard et al. 2011). Such conspicuous traits and displays usually play a role

Table 2.—Correlations (*r*) between raw variables and the six components from the PCA.

Variables	Principal Components					
	PC1	PC2	PC3	PC4	PC5	PC6
Weight	0.93	-0.14	0.04	0.13	0.3	0.04
Cephalothorax width	0.89	-0.12	0.22	0.31	-0.18	-0.03
Front leg 1	0.93	-0.20	-0.20	-0.16	0.003	-0.15
Front leg 2	0.93	-0.15	-0.21	-0.13	-0.12	0.14
White patch	0.62	0.66	0.31	-0.27	-0.00	0.004
% of white patch cover	0.17	0.93	-0.26	0.20	0.002	-0.01

in sexual selection and mate choice (Andersson 1994). Both visual (Huber 2005; Uhl & Elias 2011), and vibratory displays (Elias et al. 2004, 2005, 2006, 2010; Sivalinghem et al. 2010) are used by female jumping spiders to assess potential males for mating during courtship. These display characteristics typically convey male condition, which may influence brood survival and success (Uhl & Elias 2011). For another salticid species, *Habronattus pyrrithrix* (Chamberlin, 1924), male coloration is related to diet (Taylor et al. 2011), and males without sexual displays are not chosen by females (Taylor & McGraw 2013, but see Taylor et al. 2014). Vibratory signals are also important in female choice in the same genus (Elias et al. 2004, 2005). In *Phidippus*, another well studied genus, vibration is also important for female mate choice (Sivalinghem et al. 2010). In contrast to these results, in *H. adansoni*, no morphological character we measured, nor the white patch area or percent of white coverage were related to female response. However, we found that *H. adansoni* also exhibits vibratory signals that might be important in sexual selection, but these have not yet been explored. Moreover, although white patch area does not predict female choice, it is possible that colorimetric variables, such as reflectance in different wave lengths, play a role in sexual selection. Finally, for such multi-modal signals, it may be a combination of traits that is critical for female preference (see Girard et al. 2011). We had a big variation in age of animals in our experiments. Although we did not have a large enough sample size to add it as another factor in our models, we believe this should be focus of future studies, since age might play a role in sexual selection and mate choice.

Most of the pairs that failed to copulate did so because of female rejection. Remating of the same pair, as observed here, has been reported in other jumping spiders (Jackson & Macnab 1989a,b). Females usually determine the end of remating by not accepting further attempts by a particular male. Long copulation durations have been suggested as a strategy of mate guarding in other spiders. Since monogamy is rare in spiders (Schneider & Andrade 2011), and first sperm priority is common (Huber 2005), males may try to prolong copulations (Huber 2005; see also Drengsgaard & Toft 1999), which may partly explain the high copulation rates observed in this study. In the field, males and females have territories with very little overlap (personal observation), which may select for both sexes to engage in copulation multiple times and for long durations if the encounter rates are low in natural populations.

This is the first study to describe the breeding behavior of *H. adansoni* in detail and, as expected for a jumping spider, male courtship was complex and involved multimodal features. Morphological traits did not predict male mating success, and future work should focus on the vibratory display and reflectance of the white patch to fully understand female mate choice in this species.

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Fitness effects of nuptial gifts in the spider *Pisaura mirabilis*: examination under an alternative feeding regime

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Abstract. Nuptial feeding has variable effects on fitness within a species, partly driven by variation in female diet. We investigate nuptial feeding in the spider *Pisaura mirabilis* (Clerck, 1757) under a feeding regime that has not been explored: starvation after mating and gift consumption. We vary gift size and gift number to examine the effects on mating behavior and components of female fitness. With regard to gift size, copulation duration increased with larger gift size, but no component of female fitness was affected (time to oviposition, egg sac mass, female lifespan). These results corroborate other examinations of gift size in *P. mirabilis*. Given a likely male benefit (prolonged copulation) for larger gift size and no demonstrated female benefit, sexual conflict stands as a likely explanation for male benefits due to large nuptial gift size. With regard to gift number, components of female fitness were not affected by the consumption of one or two extra gifts. This agrees with other studies, although we note that some experiments have found the consumption of extra gifts to increase female fitness. As for males, they were more likely to copulate when they had gifts, as in other studies. We conclude some support for sexual congruence with regard to gift number, as males and females stand to benefit simultaneously from the mere presence of the gift, and females might benefit from the consumption of multiple gifts. Thus, both sexual conflict and sexual congruence appear to be at work regarding the evolution of nuptial gifts in *Pisaura mirabilis*.

Keywords: Congruence hypothesis, nuptial feeding, sexual conflict

Nuptial feeding, a male's provision of nourishment to his mate, occurs in many arthropods (Thornhill & Alcock 1983; Zeh & Smith 1985; Parker & Simmons 1989; Simmons & Parker 1989; Boggs 1995; Vahed 1998, 2007), as well as some vertebrates (Tryjanowski & Hromada 2005; Mougeot et al. 2006). This nourishment (nuptial gift) has been observed in various forms, including food items captured by the male, glandular secretions by the male, edible spermatophores, and even the male's body parts (reviewed in Gwynne 1997; Vahed 1998, 2007). Two classes of hypothesis have been proposed for the evolution of nuptial feeding: sexual congruence and sexual conflict. Sexual congruence posits that nuptial feeding offers some combination of direct and indirect benefits to the male and female (Gwynne 2008; Ortíz-Jiménez & Castillo 2015). In contrast, sexual conflict posits that nuptial feeding manipulates the behavior of one sex (typically the female), thereby providing fitness benefits to the manipulator sex (typically the male) and little to none to the manipulated sex (Sakaluk 2000; Arnqvist and Rowe 2005; Vahed 2007). Thus, to distinguish between these hypotheses, one must determine how nuptial feeding affects male and female fitness.

Research over the past few decades points to male mating benefits via nuptial feeding, as well as potential male paternal investment (Gwynne 1997; Vahed 1998, 2007). Nuptial feeding shows variable effects on female fitness (Boggs 1995; Vahed 2007; Lewis & South 2012). While many studies have demonstrated nuptial feeding to increase components of female fitness, including the number and size of eggs (e.g., Gwynne 1984, 1988; Simmons 1990; Simmons et al. 1993; Wedell 1996; Wiklund et al. 1998; Voigt et al. 2005; Engqvist 2007; Immonen et al. 2009; DiRienzo & Marshall 2013), numerous studies have failed to detect such increases in female fitness (e.g., Wedell & Arak 1989; Heller & Reinhold 1994; Will & Sakaluk 1994; Vahed & Gilbert 1997; Perry & Rowe 2008;). Such differences in results between studies present challenges when attempting to distinguish between competing hypotheses on nuptial feeding.

Differences between studies on nuptial feeding's effect on female fitness may be attributable to interspecific differences in nuptial gift composition and female metabolic and feeding rates (Boggs 1995; Vahed 1998; Jarrige et al. 2015). To evaluate a hypothesis regarding the evolution of nuptial gifts, then, it may be more appropriate to remove interspecific differences by restricting one's examination to processes within a species. Within a species, however, the effect of nuptial feeding has been found to be variable, with nuptial gifts increasing fitness components for food-limited, but not well-fed, females (e.g., Steele 1986; Boucher & Huignard 1987; Butlin et al. 1987; Fox 1993; Immonen et al. 2009). Because an arthropod's metabolism and utilization of nutrients are influenced by its feeding regime (Slansky & Scriber 1985; Slansky & Rodriguez 1987; Mayntz & Toft 2006; Toft et al. 2010), assessing the effects of nuptial feeding on fitness for a given species should include a broad array of feeding regimes for the females.

The spider *Pisaura mirabilis* (Clerck, 1757) stands as a model system in which to test the robustness of emerging patterns regarding nuptial feeding and fitness, as a solid body of literature on nuptial feeding exists for this species (reviewed in Nitzsche 2011; Ghislandi et al. 2014), and the number and size of nuptial gifts can be easily manipulated. In *P. mirabilis*, the male typically wraps a prey item in silk, carries it with the chelicerae, and presents it to the female (Bristowe 1958; Austad & Thornhill 1986; Nitzsche 1988). The female may accept the nuptial gift item, and insemination occurs while the female eats. Nuptial gifts have been found to consistently benefit the male by increasing the likelihood of mating (Stålhandske 2001; Albo et al. 2011b; summarized in Table

1), with larger gifts prolonging mating duration (Stålhandske 2001; Bruun et al. 2004; summarized in Table 1a). Whether sexual congruence or conflict is more applicable to nuptial feeding in *P. mirabilis*, then, depends on how female fitness is affected.

Empirical results on the effects of nuptial gifts on female fitness are mixed in *P. mirabilis*. Several fitness components have been examined, including time to oviposition, egg number, and egg hatching success (Stålhandske 2001; Albo et al. 2011b, 2013; Tuni et al. 2013; Toft & Albo 2015; summarized in Table 1). While an individual male does not present multiple gifts to the female during a mating encounter, females may encounter successive males in nature (Austad & Thornhill 1986), and therefore may accrue successive gifts. In some studies (Albo et al. 2011b, 2013), the experimental provision of one extra gift item to the female increased one component of fitness (i.e., egg hatching success), but not other components such as egg number (Table 1b). Stålhandske (2001) found the provision of one extra gift did not increase several measures of female fitness (i.e., time to oviposition, number of eggs in first egg sac, total fecundity). Toft & Albo (2015) found that increasing the daily number of gifts over a period of exposure to males decreased time to oviposition and increased egg number. In other studies, some effects of gift number were revealed under relatively restricted feeding regimes. In Tuni et al. (2013), females that consumed two extra gifts had higher egg hatching success when maintained on one meal per week (Table 1b). This effect of two extra gifts was not seen in females that were maintained on two meals per week. Comparing results across studies reveals a similar effect. For females that were maintained on three prey items per week, one extra gift increased copulation duration (Albo et al. 2011b), while one extra gift failed to affect copulation duration in females that were maintained on a more food-abundant regime of seven prey items per week (Stålhandske 2001). We further note that examinations of the effect of gift size on fitness components have been conducted on females maintained on six or seven prey per week, with no effect on female fitness being detected (Stålhandske 2001; Bruun et al. 2004; Table 1a). As indicated for gift number, we ask whether effects of gift size on female fitness might be revealed under a more restricted feeding regime.

Given these indications that a more restricted feeding regime can unmask fitness effects of nuptial gifts in *P. mirabilis,* we examine nuptial feeding under a regime that involves starvation after copulation and gift consumption. We examine this feeding regime for several reasons. First, the starvation period focuses on the effect of the male-donated gift *per se,* removing the possible confounding influence of background feeding after the consumption of the gift, as noted by Toft & Albo (2015). The goal of the starvation period is to standardize recent feeding to first oviposition (Maxwell 2000; Barry et al. 2008), with the nuptial gift serving as the female's last feeding opportunity before oviposition. Second, a regime that includes severe food limitation adds to the ecological scope of nuptial feeding studies in *P. mirabilis*. At some point after mating, food limitation appears to be common and rather extreme, as females do not feed while carrying egg sacs in nature (Austad & Thornhill 1986; P. Prokop, personal observations), although we acknowledge that it is not clear as to how long after mating starvation begins. Third, this regime tests the robustness of previous examinations of nuptial feeding on fitness, all of which allowed for continued feeding after copulation and gift consumption (Table 1). Exploration of an alternative feeding regime will help to test the robustness of emergent patterns in the literature on this species.

The present study examines the effects of varying gift size and gift number on components of male and female fitness that have been examined in *P. mirabilis,* such as copulation duration, time to oviposition, egg sac mass, and female lifespan. Our examination under a rather extreme feeding regime (i.e., starvation after copulation) contributes towards a more comprehensive assessment of the effects of nuptial feeding in this species. A demonstration of consistent benefits to males and females would support the sexual congruence hypothesis for the evolution of nuptial feeding. Consistent male benefits and a lack of female benefits would support the sexual conflict hypothesis. In addition to examining these alternate hypotheses, this study's approach adds to the growing recognition of the importance of study replication (Kelly 2006; Jasny et al. 2011; Makel et al. 2012; Dal-Ré et al. 2016), through which the robustness of conclusions drawn from empirical studies can be evaluated.

METHODS

General methods: spider collection, maintenance, and mating trials.—For both experiments, we captured over 170 juvenile and subadult spiders from mixed woodlands near Trnava, Slovakia in April–May 2009 (Experiment 1, gift size) and April 2011 (Experiment 2, gift number). In both years, we isolated the spiders in ventilated 0.3-L glass jars, maintained at 20°C, provided with wet cotton, protected from direct sunlight and checked daily for adult emergence. We fed the spiders cricket nymphs (*Gryllus assimilis*) three times per week (c. five crickets per feeding), and misted them with water once per day. These crickets were also used as nuptial prey items in both experiments. For Experiment 1, 87 females and 85 males molted into adulthood in May 2009; for Experiment 2, 85 females and 182 males molted into adulthood in May 2011. We fed adult males cricket nymphs *ad libitum* throughout both experiments. For adult females, we fed each female *ad libitum* for the first 4–11 days after adult emergence.

We followed the following common protocol for mating trials in both experiments. Adult males and females were virgin and sexually inexperienced. Males were 10–15 days as adults at the mating trial. Females were 12–13 days (Experiment 1) or 6–9 days (Experiment 2) as adults at their first mating trial. Two days prior to each female's first mating trial, the female was starved to encourage mating behavior, as starved or food-limited *P. mirabilis* females are more likely to mate than satiated females (Bilde et al. 2007; Prokop & Maxwell 2009). Before the trial, we anesthetized the male and female with CO_2 and measured them for body mass (to 0.0001 g) and prosoma width (to 0.01 mm). We quantified body condition as the residual values of a linear regression of body mass on prosoma width (Jakob et al. 1996; Prokop & Maxwell 2012). We conducted each mating trial in a glass terrarium (30×20×20 cm), observed by the same experimenter (PP). We placed the female inside the terrarium and allowed her 30 min

Table 1a.—Review of effects of nuptial gift size on components of fitness in *Pisaura mirabilis*. "Positive" and "Negative" refer to the effect of increasing gift size on the variable in question. "No": no effect detected. "—": effect not measured. In column headers, "cop" = "copulation."

Adult ♀ feeding regime											
Before cop	After cop	Treatments	Occurrence of cop	Cop duration	Time to oviposition	Egg number	Egg sac mass	Egg hatching success	Total fecundity	Female lifespan	Reference
1 cricket/day	1 cricket/day	No, Small, Medium, or Large gift (range = 2.3 – 29.1 mg/gift)	No	Positive	No	No	–	–	No	–	Stålhandske (2001)
6 flies /week	6 flies /week	Small or Large gift (range = 5 47 mg/gift)	–	Positive			–	–	–	–	Bruun et al. (2004)
Ad libitum crickets	Starved	Small gift (30 mg/gift) Large gift (100 mg/gift)	No	Positive	No	–	No	–	–	No	This study

to habituate and to produce drag lines while walking. This procedure is important to elicit male courtship (Lang 1996; Stålhandske 2001). We then placed the male c. 10 cm in front of the female. All males showed courtship behavior after 1–5 min, which included touching the females' drag lines, trembling of the palps and abdomen, jerking of the body, and rapid rubbing of the legs (Lang 1996). Once a male exhibited courtship behavior, we placed one gift item (freshly killed cricket nymph, *G. assimilis)* near him in Experiment 1 and for "Gift" (G) trials in Experiment 2. Upon the addition of the gift item, the male typically seized it immediately.

We recorded the following behaviors to the nearest 1 min, from the time of the male's introduction: male's wrapping of the gift with silk (binary measure – whether silk wrapping occurred), female's acceptance of the gift, occurrence of copulation (binary measure – whether at least one pedipalp insertion occurred), and beginning and ending of a given pedipalp insertion. We defined copulation as the insertion of the male's pedipalps into the female's reproductive opening (Foelix 1996). We defined copulation duration as the total amount of time spent by the male in pedipalp insertions (Stålhandske 2001; Albo et al. 2011b; Tuni et al. 2013), as

Table 1b.—Review of effects of number of nuptial gifts on components of fitness in *Pisaura mirabilis*. "Positive" and "Negative" refer to the effect of an increase in number of gifts on the variable in question. "No": no effect detected. "—": effect not measured. In column headers, "cop" = "copulation."

Adult ♀ feeding regime											
Before cop	After cop	Treatments	Occurrence of cop	Cop duration	Time to oviposition	Egg number	Egg sac mass	Egg hatching success	Total fecundity	Female lifespan	Reference
1 cricket/ day	1 cricket/day	0 or 1 gift (range = 2.3 – 29.1 mg)	Positive	No	No	No	–	–	No	–	Stålhandske (2001)
3 flies/week	3 flies/week	0 or 1 gift (low to high quality)	Positive	Positive		No	–	Positive	–	–	Albo et al. (2011b)
3 flies/ week	1 fly/day	0 or 1 gift	–	–	–	–	–	Positive	–	–	Albo et al. (2013)
2 feedings/ week	2 feedings/ week	1 or 3 gifts	–	Positive	Negative	No	–	No	No	No	Tuni et al. (2013)
1 feeding/ week	1 feeding/ week	1 or 3 gifts		Positive	Negative	No	–	Positive	No	No	Tuni et al. (2013)
2 flies/ week	see Treatments	1 prey /day; 2 prey /day[1]	–[2]	–	Negative	Positive	–	Negative	–	–	Toft and Albo (2015)
Ad libitum crickets	Starved	0, 1, or 2 gifts (10 mg/ gift)	Positive	No	No	–	No	–	–	No	This study

[1] In Toft & Albo (2015) Experiment 1, females were fed two flies per week until exposure to males. Then, females were exposed to males daily until egg sac construction. During the period of exposure to males, females were provided with one prey item per day (nuptial gift = fly), or two prey items per day (nuptial gift plus one extra fly).

[2] Females that received one prey per day showed higher number of copulations than females that received two prey per day. This is a different measure of "Occurrence of copulation" than in the other studies, which examined whether copulation occurred or not.

males may perform multiple insertions during an encounter with a female. We allowed the male and female to interact for 120 min after the male's introduction, after which time we returned them to their housing jars. We fed the males *ad libitum* until the conclusion of the trials in late May 2009 or 2011, after which we released them at their sites of capture.

For Experiment 1, we conducted mating trials between 7–22 May 2009; for Experiment 2, mating trials were between 26 April–4 May 2011. During a given day, we conducted up to ten separate trials simultaneously. Mating trials began during 0800–0900. To visually isolate male–female pairs, we attached white paper to the external sides of each terrarium. We randomly assigned the order of treatments within a day. After a female's mating trial(s), we returned her to her housing jar to lay egg sacs (egg sacs weighed to nearest 0.0001 g). We daily sprayed the females with water, but did not offer them food until oviposition.

Experiment 1: effect of gift size.—At each mating trial, we paired the virgin female with a virgin male who was randomly assigned to one of two gift treatments: large gift (male given a cricket nymph of 0.10 g; $n = 40$ trials) or small gift (male given a cricket nymph of 0.03 g; $n = 35$ trials). Neither male nor female body measurements (body mass, prosoma width, body condition) significantly differed between treatment groups (*t*-tests: all $t_{73} < 1.95$, all $P > 0.05$).

Experiment 2: effect of gift number.—To vary the number of gifts offered to a female, we paired each female with two virgin males in succession. Female polyandry in nature is indicated for *P. mirabilis* by Austad & Thornhill (1986), and previous experimental studies have presented successive males to females (Drengsgaard & Toft 1999; Prokop & Maxwell 2009; Tuni & Bilde 2010; Tuni et al. 2013; Toft & Albo 2015). For a given trial, we randomly provided the male with a cricket nymph as a gift item (Gift, G; gift size = 0.01 g) or no gift item (No gift, N). We randomly assigned each female to one of four treatment groups: Gift-Gift (GG, $n = 22$), Gift-No gift (GN, $n = 21$), No gift-Gift (NG, $n = 19$), and No gift-No gift (NN, $n = 21$). Thus, each female was presented with either zero, one, or two gifts, while the number of encountered males was held constant. In total, we used 166 males in the trials (i.e., 2 males per female, 83 females), with each male being virgin, sexually inexperienced, and used once. All females were virgin at their first trial. In trials where the female received a gift, we standardized gift consumption by the female by returning the gift item to the female in cases where the male escaped with it before it was eaten by the female. After the first trial, the female remained in the mating terrarium. The second trial began with the introduction of a new male c. 60 minutes after the end of the first trial.

Female body measurements (body mass, prosoma width, body condition) did not significantly differ among the four treatment groups (ANOVA tests: all $F_{3,79} < 1.00$, all $P > 0.4$). Male body measurements did not differ among treatment groups (ANOVA main effects, treatment: all $F_{3,158} < 1.95$, all $P > 0.1$. Overall, males used in the second trial were heavier than those in the first trial (mean ± SE, body mass (g): trial 1 = 0.099 ± 0.002, trial 2 = 0.104 ± 0.002; ANOVA main effect, trial: $F_{1,158} = 5.53$, $P < 0.05$), as well as wider (mean ± SE, prosoma width (mm): trial 1 = 3.73 ± 0.04, trial 2 = 3.86 ± 0.04; ANOVA main effect, trial: $F_{1,158} = 6.53$, $P < 0.05$). Males did not differ in body condition between trials (ANOVA main effect, trial: $F_{1,158} = 0.05$, $P = 0.83$).

Statistical analyses.—We analysed the effects of gift size (Experiment 1) and gift number (Experiment 2) on various mating behaviors and components of female fitness. For continuous dependent variables (time to copulate, copulation duration, time to first oviposition, egg sac mass, female lifespan), analysis was through parametric tests. For dichotomous dependent variables (occurrence of copulation, occurrence of egg laying), analysis was through logistic regression. Statistical tests were performed with Statistica (Version 8, StatSoft 2007, Tulsa, OK, USA, online at http://www.statsoft.com). We assessed normality via Shapiro-Wilks test. For time to copulate, we achieved normality via Box-Cox (x+0.1) transformation. All tests are two-tailed.

RESULTS

Experiment 1: effect of gift size.—Upon being presented with a prey gift item, 74 of 75 males seized it. Of the males that took the small gift, 54% wrapped it (19 of 35 males); 49% of males that took the large gift wrapped it (19 of 39 males). Gift size did not significantly affect whether a male wrapped the gift item (Logistic regression: Wald's $\chi^2 = 0.34$, $P = 0.56$). For the 74 trials in which the male seized the gift item, 56 trials resulted in gift consumption and successful copulation, and 15 trials did not result in copulation (8 trials without copulation in the large gift treatment, 7 trials without copulation in the small gift treatment). Three trials resulted in the female's cannibalism of the male during copulation. Because sperm transfer was likely interrupted in these three trials, they were excluded from further analysis.

With regard to the occurrence of copulation in the 71 trials for analysis, neither gift size nor the wrapping of the gift with silk showed a significant effect (Logistic regression, main effects: Wald's $\chi^2 = 0.16$ and 0.60, $P = 0.90$ and 0.44, respectively). For 56 trials with copulation, duration was longer for males with large gifts, and for males who wrapped their gifts in silk (Figure 1; ANOVA: whole-model test $F_{3,52} = 4.09$, $P < 0.05$, gift size $P < 0.01$, gift wrapping $P < 0.05$, interaction $P = 0.1$). Time to copulate was not significantly affected by gift size or gift wrapping (ANOVA: whole-model test $F_{3,52} = 0.04$, $P = 0.99$).

With regard to female fitness, 42 of the 56 females that copulated produced one egg sac; no female produced two or more egg sacs. For the occurrence of oviposition, neither gift size nor the wrapping of the gift with silk showed a significant effect (Logistic regression, main effects: Wald's $\chi^2 = 1.99$ and 0.92, $P = 0.16$ and 0.40, respectively). We found no significant effect of gift size or gift wrapping on the time to first oviposition (ANOVA: whole-model test $F_{3,38} = 0.48$, $P = 0.69$; Table 2). We found no significant effect of gift size or gift wrapping on the mass of the first egg sac (ANOVA: whole-model test $F_{3,38} = 1.92$, $P = 0.14$; Table 2). Female lifespan was not affected by gift size, gift wrapping, or egg sac mass (multiple linear regression: adj. $R^2 = 0.05$, $F_{3,52} = 1.87$, $P = 0.17$; Table 2); this result remains when females that did not produce an egg sac are removed from analysis (multiple linear regression: adj. $R^2 = 0.01$, $F_{3,38} = 1.10$, $P = 0.34$).

Experiment 2: effect of gift number.—All males presented with a prey gift item seized it. In the first mating trials, 40 of

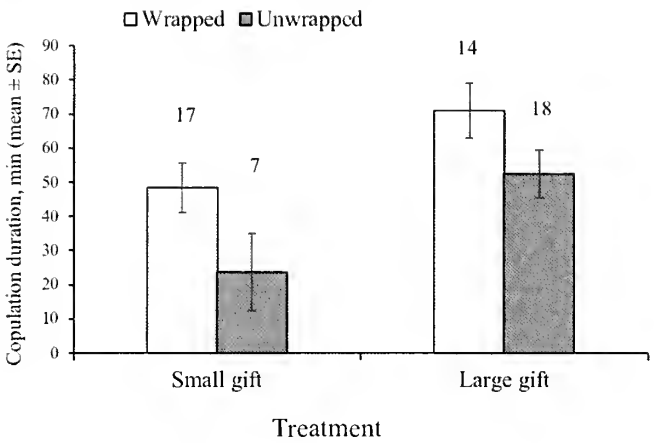

Figure 1.—Experiment 1 (gift size): copulation duration for small and large gift items, when the male wraps the gift (white bars) or does not wrap the gift (dark bars). Numbers above bars are sample sizes.

the 43 males wrapped the gift with silk (i.e., trial 1 of GG and GN treatments); in the second mating trials, 38 of the 41 males wrapped the gift (i.e., trial 2 of GG and NG treatments). Exclusion of the six males that did not wrap the gifts did not affect the statistical significance of the following results. Looking at only the first mating trial, where both the male and female were sexually naive, all of the males with gift items successfully copulated (43/43 = 100%; trial 1 of GG and GN treatments), whereas only 28% of the males without gifts copulated (11/40 = 28%; trial 1 of NG and NN treatments; Table 3; Fisher exact: $P < 0.001$).

Overall, 68 of the 83 females copulated at least once (Table 3). Each copulation involved the consumption of the gift item by the female. The number of copulations differed among the treatment groups (Table 3). Average number of copulations was highest for females that were presented with two successive males bearing gifts (GG treatment; ANOVA: whole model test $F_{3,79} = 58.17$, $P < 0.001$). These results were statistically equivalent when males that did not wrap the gifts were excluded from analysis (ANOVA: whole model test $F_{3,79} = 32.73$, $P < 0.001$). Females in trials with males without gifts often did not assume the typical mating position (i.e., elevated opistosoma), but rather remained with their opistosoma close to the ground, thereby blocking the males' insertions of the pedipalps into the epigyne.

Overall mean time to copulate was 13 min (SE = 1 min, n = 99 trials with copulation). Gift number did not affect time to copulate. For females presented with two gifts (GG treatment), time to copulate did not differ between trial 1 (first gift presented; mean ± SE = 15 ± 2 min, $n = 22$ trials) and trial 2 (second gift presented; mean ± SE = 16 ± 2 min, $n = 22$ trials; paired t-test: $t_{21} = 0.4$, $P = 0.75$). Across treatments, females presented with one gift (trial 1 in GN; mean ± SE = 12 ± 2 min, $n = 21$ females), one gift after previous exposure to a male (trial 2 in NG, mean ± SE = 10 ± 2 min, $n = 19$ females), or two successive gifts (trial 2 in GG) did not differ in time to copulate (ANOVA: whole model test $F_{2,59} = 2.29$, $P = 0.15$). Similarly, gift number did not affect copulation duration. Overall mean copulation duration was 46 min (SE = 3 min, n = 99 trials with copulation). For females presented with two gifts (GG treatment), copulation duration did not differ between trial 1 (first gift presented; mean ± SE = 46 ± 6 min, $n = 22$ trials) and trial 2 (second gift presented; mean ± SE = 55 ± 6 min, $n = 22$ trials; paired t-test: $t_{21} = 1.26$, $P = 0.27$). Across treatments, females presented with one gift (trial 1 in GN; mean ± SE = 55 ± 6 min, $n = 21$ females), one gift after previous exposure to a male (trial 2 in NG; mean ± SE = 55 ± 6 min, $n = 19$ females), or two successive gifts (trial 2 in GG) did not differ in copulation duration (ANOVA: whole model test $F_{2,59} = 0.002$, $P = 0.98$).

Table 2.—Experiment 1 (gift size): female reproductive output and lifespan (mean ± SE).

Treatment	Time to oviposition (days)	Egg sac mass (g)	Lifespan (days)
Large gift ($n = 26$)	29.1 ± 1.3	0.0390 ± 0.0055	84.7 ± 2.8
Small gift ($n = 16$)	29.8 ± 1.8	0.0402 ± 0.0070	78.3 ± 4.1

Four females cannibalized males during trial 1 or 2 (two females in GG, two females in NN). To focus on the effect of gift consumption on female fitness, these females were removed from further analysis. Over all treatments, twenty females produced an egg sac (Table 4); no female produced two or more egg sacs. The number of gifts consumed did not affect the occurrence of egg laying (Logistic regression: Wald's $\chi^2 = 3.02$, $P = 0.27$). For the 20 females that layed eggs, the number of gifts consumed did not affect the time to oviposition (linear regression: $R^2 = 0.004$, $F_{1,18} = 0.08$, $P = 0.74$), or the mass of the egg sac (linear regression: $R^2 = 0.01$, $F_{1,18} = 0.23$, $P = 0.66$). Female lifespan was not affected by the number of gifts consumed or the occurrence of egg laying (multiple linear regression: $R^2 = 0.015$, $F_{2,80} = 0.62$, $P = 0.57$). To examine whether the mass of the egg sac affected female lifespan, we removed females that did not produce an egg sac from analysis, and included egg sac mass as an independent variable. An overall significant effect resulted, but neither independent variable emerged as a significant effect on female lifespan (multiple linear regression: $R^2 = 0.32$, $F_{2,17} = 3.95$, $P < 0.05$; number of gifts $P = 0.07$; egg sac mass $P = 0.06$).

Table 3.—Experiment 2 (number of gifts): copulations per female. Females were presented with two consecutive males that were bearing a gift (G) or without a gift (N). GG = Gift-Gift; GN = Gift-No gift; NG = No gift-Gift; and NN = No gift-No gift. Thus, each female was presented either zero, one, or two gifts, while number of encountered males was held constant. Treatments with different letters differ significantly (ANOVA followed by Tukey HSD post-hoc test; $p < 0.05$).

		# females that copulated			# copulations/ female
Treatment	# females	Trial 1 only	Trial 2 only	Trial 1 & 2	(mean ± SE)
GG	22	0	0	22	2.00 ± 0.00 a
GN	21	21	0	0	1.00 ± 0.00 b
NG	19	0	12	7	1.37 ± 0.11 c
NN	21	2	2	2	0.38 ± 0.15 d

Table 4.—Experiment 2 (number of gifts): female reproductive output and lifespan (mean ± SE). Females were presented with two consecutive males that were bearing a gift (G) or without a gift (N). Abbreviations as in Figure 3.

Treatment	Time to oviposition (days)	*n*	Egg sac mass (g)	*n*	Lifespan (days)	*n*
GG	9.7 ± 3.4	3	0.0144 ± 0.0125	3	50.8 ± 2.1	22
GN	8.2 ± 2.7	5	0.0328 ± 0.0097	5	52.4 ± 2.1	21
NG	14.3 ± 2.1	8	0.0171 ± 0.0076	8	48.6 ± 2.2	19
NN	11.3 ± 3.0	4	0.0086 ± 0.0108	4	52.6 ± 2.1	21

DISCUSSION

Evaluation of alternate hypotheses for the evolution of nuptial feeding—sexual congruence and sexual conflict—requires an assessment of how male and female fitness are affected within a species. The present study contributes towards such an evaluation for the spider *Pisaura mirabilis*, as it corroborates several emergent patterns from the literature on male and female fitness (summarized in Table 1). Given the range of feeding regimes for the females in the various studies on *P. mirabilis*, finding consistent results across studies points to robust conclusions for this species.

With regard to gift size, copulation duration increased with larger gifts (Experiment 1), pointing to a likely male fitness benefit. This result agrees with previous examinations of gift size in *P. mirabilis* (Stålhandske 2001; Bruun et al. 2004), where copulation duration was found to increase with gift size. Longer copulation duration is associated with higher fertilization success in this species (Stålhandske 2001). When a female mates with two males, a male's fertilization success increases as his copulation duration increases relative to his rival's (Drengsgaard & Toft 1999). Gift size failed to affect other mating behaviors, such as the occurrence of copulation and time to copulation. With regard to female fitness, gift size showed no effect on the occurrence of oviposition, time to oviposition, egg sac mass, or female lifespan in the present study. These results corroborate Stålhandske (2001), who similarly failed to find an effect of gift size on time to oviposition and measures of egg output. The lack of an effect of gift size on female fitness appears to be a robust result, as females were constantly fed before and after mating in Stålhandske (2001), in contrast to the post-mating starvation in the present study.

At present, empirical studies suggest that larger gift size benefits the male by prolonging copulation, but shows no effect on female fitness. This conclusion points to sexual conflict as the more likely explanation for male benefits due to large nuptial gift size. We note that a full evaluation of this hypothesis includes costs to the male when acquiring larger gifts. Such costs are not known, but larger gifts might involve heavier transportation costs, as Prokop and Maxwell (2012) have demonstrated reduced running speed for males when carrying gifts.

Gift number showed variable effects in the present study. The number of copulations per female increased with gift number (Experiment 2), as all females exposed to two gift-bearing males copulated twice, females exposed to one gift-bearing male copulated once on average, and most females exposed to no gift-bearing males did not copulate at all. Furthermore, for the first trial of this experiment, when both the male and female were sexually inexperienced, males with gifts were significantly more likely to mate than males without gifts. This result is consistent with other studies that indicate that the presence of a gift is an important determinant of mating success in *P. mirabilis* (Austad & Thornhill 1986; Stålhandske 2001; Prokop 2006; Prokop & Maxwell 2009; Nitzsche 2011; Albo et al. 2011b, 2013). Thus, possessing a gift increases a male's chance of mating. Recent work points to another male benefit of gift possession: reduced risk of cannibalism. In Toft & Albo (2016), males with gifts were less likely to be cannibalized by the females than were males without gifts.

The present study did not find effects on female fitness for gift number. Gift number failed to affect the occurrence of oviposition, time to oviposition, egg sac mass or female lifespan. These results agree with some studies that have examined these fitness components in *P. mirabilis*, thereby allowing some conclusions to be drawn (summarized in Table 1b). In Stålhandske (2001), the provision of one extra gift did not affect time to oviposition, the number of eggs in first egg sac, or total fecundity. In Albo et al. (2011b), the provision of one extra gift did not affect clutch size or the number of spiderlings per egg sac. In Tuni et al. (2013), the provision of two extra gifts did not affect egg number or female lifespan, but the two extra gifts decreased time to oviposition. From these studies, gift number consistently fails to affect total fecundity and female lifespan (Stålhandske 2001; Tuni et al. 2013; this study).

For other measures of female fitness, the effects of gift number are complicated (Table 1b). For egg number and egg sac mass, the literature approaches a consensus in that most studies do not find an effect of gift number on either measure (Stålhandske 2001; Albo et al. 2011b; Tuni et al. 2013; this study). An exception is Toft & Albo (2015), in which females were placed on two feeding regimes in one of the study's experiments. Females were supplied with either one or two prey items per day, and encountered a male daily. Females supplied with two prey per day laid more eggs than females supplied with one prey per day. In other studies, an effect on female fitness may emerge after a certain number of gifts have been consumed. For example, time to oviposition does not appear to be affected when one extra gift item is consumed (Stålhandske 2001). Time to oviposition can decrease when two or more extra gifts are consumed (Tuni et al. 2013; Toft & Albo 2015), although we caution that we failed to find such an effect in the present study, where females consumed one or two extra gifts. Still other fitness measures remain difficult to evaluate. These include egg hatching success, where various studies report positive (Albo et al. 2011b, 2013), negative (Toft

& Albo 2015), or no effect (Tuni et al. 2013) of the number of nuptial gifts.

The varied effects of gift number on components of female fitness point to sexual congruence as the more likely explanation of benefits derived from one or more gift. In the simplest case, a male with a gift is more likely to mate, and less likely to be cannibalized, than a male without a gift (Austad & Thornhill 1986; Stålhandske 2001; Prokop 2006; Prokop & Maxwell 2009; Nitzsche 2011; Albo et al. 2011b, 2013; Toft & Albo 2016; this study; Table 1b). A female undoubtedly derives nutrients from a single gift, but significant fitness effects might not be seen until she consumes multiple gift items, as suggested by some results for time to oviposition (Stålhandske 2001; Tuni et al. 2013; Toft & Albo 2015; but see this study; Table 1b). We caution that these benefits to males and females need to be weighed against the possible costs and constraints involved. For males, gift construction requires time spent foraging, expenditure in silk, and probable transportation costs (Albo et al. 2011a; Nitzsche 2011; Prokop & Maxwell 2012; Ghislandi et al. 2014). For females, the reproductive benefits of consuming multiple gifts might plateau or decrease after a certain number, as suggested by Toft & Albo (2015).

An unexpected result in the present study was the lower incidence of gift wrapping by males in Experiment 1 (51% of males that took the gift) than by males in Experiment 2 (93% of males that took the gift). A likely explanation lies in the sizes of the gifts in the two experiments. The gifts in Experiment 2 were smaller (0.01 g) than those in Experiment 1 (0.03 and 0.10 g). Males given the small gifts in Experiment 2 may have wrapped the gifts to increase their size or volume (Ghislandi et al. 2014; Prokop & Semelbauer 2017). It is likely that females spend more time manipulating wrapped gifts, which appears to contribute to longer copulation durations. Indeed, gift wrapping increased copulation duration in Experiment 1, as in Lang (1996) and Andersen et al. (2008). Given that longer copulations correlate with higher fertilization success (Drengsgaard & Toft 1999; Stålhandske 2001), gift wrapping evidently confers fertilization benefits to males. Furthermore, gifts wrapped with silk in nature are more frequently lighter and worthless (i.e., containing inedible prey) than unwrapped gifts (Ghislandi et al. 2014; Prokop & Semelbauer 2017), suggesting that males may mask the content of low-quality gifts through wrapping.

In sum, both hypotheses appear to be at work regarding the evolution of nuptial gifts in *Pisaura mirabilis*. Males and females both stand to benefit from the mere presence of the nuptial gift: the male is more likely to mate and the female obtains nutrients. This outcome supports the sexual congruence hypothesis. Females may benefit from the consumption of multiple gifts, but results are mixed, as multiple feeding positively affects some fitness components while not affecting others. With regard to gift size, males prolong copulation, and therefore likely increase fertilization success, by providing larger gifts to their mates. Females, on the other hand, do not appear to benefit by consuming larger gifts. This outcome supports male benefits via larger gifts as having arisen through sexual conflict.

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Estimating biomass from body size of European spiders based on regression models

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Abstract. There is a need for reliable and standardized methods to measure functional species traits. Body mass is just one dimension of body size, a most important morphological trait, because it is directly linked with metabolic rate and affected by environmental conditions. However, it is still not widely used due to the difficulties and constraints of direct measures. Weighing many (small) animals (i.e., arthropods) is laborious, time consuming and biased when using preserved material. Therefore, the applicability of general equations for estimating mass from body size of spiders was tested. We calculated linear regressions to estimate fresh and dry mass of spiders from different body measures (i.e., body length, carapace length and width) of 189 spiders sampled in southern Germany. We compared these regressions with each other and with equations from the literature and tested the impact of taxa, sex and habitat on the accuracy of biomass estimates using an independent test dataset of 166 spiders. All size-fresh mass regressions were highly significant with R^2 values between 0.81 and 0.98. The slope of the ln-transformed body mass - body size relationship ranged between 2.51 and 2.95. The regressions including total body length always showed higher R^2 values, i.e., they provide better predictions of body mass than carapace measures. The body length-dry mass regression was also highly significant and the mean ratio dry mass/fresh mass was 0.22. Taxon-, sex- or microhabitat-specific regressions did not produce better estimates than general regressions. Therefore, we strongly recommend the use of general regressions in the context of biomass estimation of assemblages and propose parameters from our regressions to be used for European spiders.

Keywords: Araneae, morphological traits, practical use, fresh mass

Biomass is an important parameter in population and community studies because it is directly related to nutrient and energy availability at each trophic level in all ecosystems (Vucic-Pestic et al. 2010; Chapin et al. 2012). It is also an important morphological and functional trait, correlated with the individual's metabolic rate and food requirement (Peters 1983; Hudson et al. 2013), scaling with many life-history traits (Moretti et al. 2016). Body size and mass are determinants of the interactions of any organism with its abiotic and biotic environment. Both are quantitative and measurable under standardized conditions and therefore among the traits recommended for terrestrial invertebrates (Moretti et al. 2016).

Directly determining the mass of living arthropods by weighing is technically elaborated, laborious, time consuming and error-prone. In ecological studies, spiders and other arthropods are usually caught in different preserving agents and transferred to ethanol for identification and voucher depositing, causing unpredictable changes in fresh mass and prohibiting desiccation for dry mass. Therefore, alternative methods to estimate the body mass have repeatedly been developed and tested (e.g., for aquatic invertebrates: Johnston & Cunjak 1999; Miserendino 2001; Sabo et al. 2002; for terrestrial invertebrates: Rogers et al. 1976, 1977; Sage 1982; Sample et al. 1993; Hódar 1996; Wardhaugh 2013). The most common and approved method is to calculate statistically tested regressions of mass and body size measures. Although body volume would be the logical predictor of mass (Moretti et al. 2016) direct measures of volume or the body diameter are rather difficult to take. Therefore, calculations of fresh or dry mass based on easily measured body size dimensions such as length or width are clearly preferable. Previous publications often propose the power model as the best model to describe the size-mass relationships (Breymeyer 1967; Rogers et al. 1976; Gowing & Recher 1984; Ganihar 1997; Brady & Noske 2006; Höfer & Ott 2009; Martin et al. 2014), but often use it in its linearized form (Ganihar 1997; Wardhaugh 2013). Linear models based on logarithmic data have also been used to describe the relationship (Rogers et al. 1977; Ganihar 1997; Höfer & Ott 2009).

Spiders are a diverse group and although their bodies' physical structure is rather uniform (i.e., oval prosoma and opisthosoma, chitinized carapace) across all developmental stages and life forms, body shapes vary, e.g., in the ratio prosoma-opisthosoma or the ratio body-leg size. It is therefore important to know if regressions of body size-mass across multiple species (herein called general equations) can be used for all spider assemblages, at least in the same biogeographical/climatic region. In Central Europe, there are very few extreme shapes, as for example in ant-mimic spiders (*Synageles* Simon, 1876, *Myrmarachne* MacLeay, 1839) or the mygalomorph *Atypus* Latreille, 1804 with their compact legs and big chelicerae. Within a species, body size and mass (of adults) can vary considerably and not only by sexual dimorphism (Mikhailov 1996; Foellmer & Moya-Laraño 2007; Wunderlich 2008; Logunov 2011). Size (and mass) of adults depend on their sexual maturity, i.e., the developmental stage of eggs in females. Ecological factors causing size and mass variation are nutritional conditions during the development and life cycle (Jocqué 1981a; Jakob et al. 1996), related to climatic-geographical (Entling et al. 2010; Bowden et al. 2013) and habitat conditions (e.g., microclimate, structure, disturbance; Jocqué 1981a, b). Deviations of the size-mass relationship, i.e., caused by different densities of body mass could result from extraordinarily strong chitinization, occurring in several families (e.g., Atypidae, Corinnidae, Thomisidae, Araneidae, Linyphiidae, Theridiidae), or from opisthosoma content (eggs, guanine), but is expected to be low in comparison with the existing size variation.

Although the necessary accuracy or tolerable error of a biomass estimate certainly depends on the scientific question, it is desirable to have reliable general equations for mass estimation by regression of body size and this seems feasible for spiders (Henschel et al. 1996; Lang et al. 1997; Höfer & Ott 2009).

The global spider community has recently been estimated to equal 25 million tons and to consume between 400 and 800 million tons of prey per year on a global level (Nyffeler & Birkhofer 2017). This study however, also shows the scarcity of available reliable and comparable biomass measures worldwide. For the global impact assessment, a total of only 65 values of spider biomass m^{-2} were gathered from the literature – for all terrestrial biomes (Nyffeler & Birkhofer 2017).

For spiders of temperate zones in North America, there are equations for fresh and dry mass from a large sample of different species (Edwards & Gabriel 1998); for Europe (Central Europe, Palearctic) there are only few general equations for (dry) mass from body length from Breymeyer (1967: 3 lycosid species); Clausen (1983: 8 species from Denmark); Henschel et al. (1996: 138 spiders of 11 species from southern Germany); Hódar (1996: 18 spiders from arid zone of southeastern Spain) and Lang et al. (1997: 17 linyphiid and 6 lycosid species from arable land).

In this study, we wanted to (1) derive body mass-size relationships from a larger and diverse sample of European spiders (creation dataset, 189 specimens), (2) identify the body size measure that predicts biomass best and (3) test if taxon, sex or microhabitat influence the mass estimation to an extent that makes the use of specific regressions necessary. The latter was done applying the parameters from the creation dataset to a second independent sample of spiders (test dataset, 166 specimens). As a result, we propose regression parameters for three body size measures to estimate biomass of Central European spider assemblages.

METHODS

Sampling.—For the calculation of the regressions, 189 spiders (creation dataset) were sampled during five collection events in the surroundings of Karlsruhe, Baden-Württemberg, Germany between 12 April and 9 May 2016. Additionally, spiders from the Swabian Alp and from Bavaria were included. Spiders were captured manually or with a modified hand-held vacuum cleaner during visual searching, by beating vegetation, sifting litter and pitfall traps in order to cover the whole range of spider types in the sampling locations (Supplementary material Table S1, online at http://dx.doi.org/10.1636/JoA-S-17-044.s9). To test the regressions, we used a second dataset of 166 spiders (test dataset) sampled within a radius of 50 km around Karlsruhe in June 2016 and April and September 2017 (forests, open areas in the Black Forest) (Supplementary Material Table S1). All spiders were captured alive and stored individually in vials for a maximum of 24 hours until weighed.

Weighing and measuring.—Specimens were anesthetized with CO_2 and weighed to the nearest 0.01 mg with a microbalance (Sartorius Supermicro S4). Subsequently, the spiders were killed and preserved in 75% ethanol. Body size measurements were taken from the preserved specimens with a micrometer eyepiece to the nearest 0.05 mm: 1. Total body length, from above, excluding chelicerae and spinnerets = BL; 2. Length of the carapace (dorsal part of the prosoma) = CL; 3. Width of the carapace = CW. Thereafter the spiders were identified, juvenile specimens at least to genus or family and all adults to species. Nomenclature is based on the World Spider Catalog (2017), version 18.0 (online at http://wsc.nmbe.ch). Specimens were deposited in the collection of the State Museum of Natural History Karlsruhe (SMNK-ARA, SMNK-STUD). The body length measurement of 30 spiders was repeated after one year's storage in 75% ethanol to check for the influence of the preservation process on the size. This effect was tested using a paired t-test. To calculate a regression for body length-dry mass in order to enable dry mass estimation and – more important – to enable comparison with equations in the literature (almost exclusively for dry mass), we dried these 30 specimens in a laboratory oven at 70 °C for 48 hours and weighed them on the same microbalance. These measurements also served to calculate a ratio of dry/fresh mass.

Regression analyses.—In preliminary tests we estimated the body mass of the 189 specimens comparing the power model ($mass = a\ (size)^b$), exponential model ($mass = a + (e)^{b(size)}$) and linear model ($\ln(mass) = a + b \ln(size)$). We selected the linear model as the best fitting one based on the residual standard errors and the R^2 values.

Linear regressions for fresh mass were then calculated (using the creation dataset, 189 spiders) for each of the following data subsets: (1) all spiders, (2) lycosids, (3) males/females, (4) ground/vegetation dwelling spiders. For each of these groups, regressions were calculated based on the size measurements mentioned above: total body length (BL), carapace length (CL), carapace width (CW) and the product of body length x carapace width (BL x CW). A regression for body length-dry mass was calculated for the 30 dried specimens. All regression analyses were performed in R 3.3.0 (R Development Core Team 2016) with the function "lm". The adjusted R^2 and the standard deviations of the residuals are presented as goodness-of-fit and regression parameters a and b for the linear model mass = exp (a + b (ln body measure)) with standard errors to be used for future estimates. Plots show the individual values, regression lines and 95% prediction intervals of the regression. In contrast to the often-used confidence interval (which is an estimate of the "true" population mean of the actual observed sample), the prediction interval uses the observed sample statistics of mean and standard deviation to estimate an interval in which future observations will fall with a certain probability. Therefore, it is more appropriate to graphically represent the range in which the regression can be used.

Comparison of the regressions.—We tested the influence of taxon, sex and microhabitat using the different regressions (1–4) to estimate the mass of the test sample (166 spiders) by:

(a) applying the parameters of the lycosid-specific (2) and the general regressions (1) to the 71 lycosid spiders of the test sample to compare the resulting estimates.
The taxon Lycosidae was selected based on a sufficient number in both samples and the fact that lycosids usually predominate the very common pitfall trap samples due to

their high activity density and comparatively large body size.

(b) applying the parameters of the sex-specific (3) and the general regressions (1) to the 76 adult spiders of the test sample to compare the resulting estimates.

(c) applying the parameters of the microhabitat-specific (4) and the general regressions (1) to the 97 ground-dwelling spiders and the 69 vegetation dwelling spiders of the test sample to compare the resulting estimates.

We used ANOVA to compare the results of the different regression models. If this test showed a significant effect, Tukey's HSD post-hoc test was applied. All ANOVA and post-hoc analyses were carried out in Statistica 9.0 (StatSoft 2009).

RESULTS

Sampling.—A total of 189 spiders was sampled, weighed and measured for the regression analyses. 120 (63.5%) spiders were adult (50 males, 70 females), 105 specimens were sampled on the ground, 83 from vegetation (herb or shrubs), one specimen was caught in a house. The sample represented 47 species of 17 families (Supplementary Material Table S1, online at http://dx.doi.org/10.1636/JoA-S-17-044.s9). Wolf spiders (Lycosidae) dominated the sample with 58 individuals (31%) of 5 genera and 10 species. Total body length of the spiders ranged from 1.15 mm (a juvenile linyphiid) to 16.8 mm (an adult female of *Eratigena atrica* (C. L. Koch, 1843)), body mass ranged from 0.25 mg to 432.8 mg (same specimens). The body shape (ratio CW/BL) was rather similar for all spiders (mean: 2.7) except for the specimens of the genus *Tetragnatha* Latreille, 1804 (4.2), sampled in vegetation (Supplementary Material Table S2, online at http://dx.doi.org/10.1636/JoA-S-17-044.s10).

Regressions.—Comparing the different regression models (BL all spiders) the linear model (using ln transformed data) showed the least residual standard error (0.31, back-transformed 1.36) in comparison to the power model (2.17) and the exponential model (2.29) and also the highest R^2 values (Linear: 0.96, Power: 0.95, Exponential: 0.93). The estimated total body mass of the 189 spiders differed by only 3% from the weighed value using linear regression, but 4% using the power model and 17% using the exponential model. Therefore, we decided to use linear regression analyses, based on ln-transformed measures of body size and mass values in order to reduce heteroscedasticity. The use of a linear regression has the strong advantage that R^2 can be used as goodness-of-fit in comparisons of the different regressions (Anderson-Sprecher 1994).

All size-fresh mass regressions (Fig. 1; Supplementary Material, Figs. S1–S5, online at http://dx.doi.org/10.1636/JoA-S-17-044.s1 through dx.doi.org/10.1636/JoA-S-17-044.s5) were highly significant ($P < 0.0001$) with R^2 values between 0.806 (CW of Lycosidae) and 0.984 (BL x CW of male spiders) and standard deviations of the residuals of 0.17–0.42 (Table 1). The regression parameters a (intercept) and b (slope) are presented in Table 2 with their standard errors. The slope parameter b ranged from 2.51 to 2.95 for regressions based on single body measures. Only two of the 24 regressions showed a fit $R^2 < 0.9$, but 13 a fit $R^2 > 0.95$.

The body length-dry mass regression was also highly significant (a = -3.1726, b = 2.6296, $R^2 = 0.91$, SD = 0.42). The ratio dry mass/fresh mass was between 0.13 and 0.34 with a mean of 0.220 (± 0.05) for the weighed values and between 0.20 and 0.23 with a mean of 0.213 (± 0.01) for the estimated values.

The repeated measurement of body length of 30 spiders after one year in 75 % ethanol resulted in a mean difference of -0.056 mm (1.2%), not significant ($t = 2.093$; $P = 0.08$, paired t-test).

Comparison of the regressions.—The application of the lycosid specific regressions showed a weakly significant effect of (a) taxon-specific regressions ($F(8, 630) = 2.234$, $P = 0.024$) for biomass estimation, but a post-hoc test showed no significant differences between single regressions (Supplementary Material Fig. S6, online at http://dx.doi.org/10.1636/JoA-S-17-044.s6).

There was no significant influence of (b) sex ($F(8, 666) = 1.110$, $P = 0.354$) or (c) microhabitat ($F(8, 1485) = 1.218$, $P = 0.284$) on the body mass estimates (Supplementary Material Figs. S7 & S8, online at http://dx.doi.org/10.1636/JoA-S-17-044.s7 and http://dx.doi.org/10.1636/JoA-S-17-044.s8).

The total dry mass of the 30 spiders was 102.4 mg. Our own dry mass regression underestimated these spiders by 14%. Regressions from literature either underestimated or overestimated the mass by 19–38% (Supplementary Material Table S3, online at http://dx.doi.org/10.1636/JoA-S-17-044.s11).

DISCUSSION

In contrast to other studies which described the power model to be best suited for most insects and spiders (Ganihar 1997; Brady & Noske 2006), the linear model was more appropriate in our study showing the least residual standard error, the highest R^2 value and the smallest difference between estimated and weighed mass. A power model is better adjusted to spiders at the upper end of the size spectrum whereas a linear model better estimates the mass of the small spiders (see Höfer & Ott 2009: Fig. 1). Using a linear model to estimate a sample including large spiders (which is more frequent in tropical assemblages) may result in a large bias of the total estimate, although the mass of most of the (smaller) spiders is well estimated. Analogous to Ganihar (1997) and Brady & Noske (2006), the exponential model was suboptimal for body mass calculation of our German data set. Because linear models show further advantages in the comparison and interpretation of graphs, we decided to use linear regression models on log-transformed data for all further regression analyses and propose this procedure and the resulting regression parameters.

Comparing the goodness-of-fit, the regressions including body length and carapace width showed the highest R^2 values, lowest standard deviations, and also the smallest prediction intervals (Table 1, Fig. 1). The regressions based on BL showed a better fit than the ones using carapace measures. This is certainly caused by the fact that the opisthosoma contributes most to the variation in size and mass within the species, depending mainly on the nutritional status and the development of the sexual organs (Jakob et al. 1996). If this variation is included, the regression becomes more precise. On the other hand, the use of a carapace measure of adult spiders

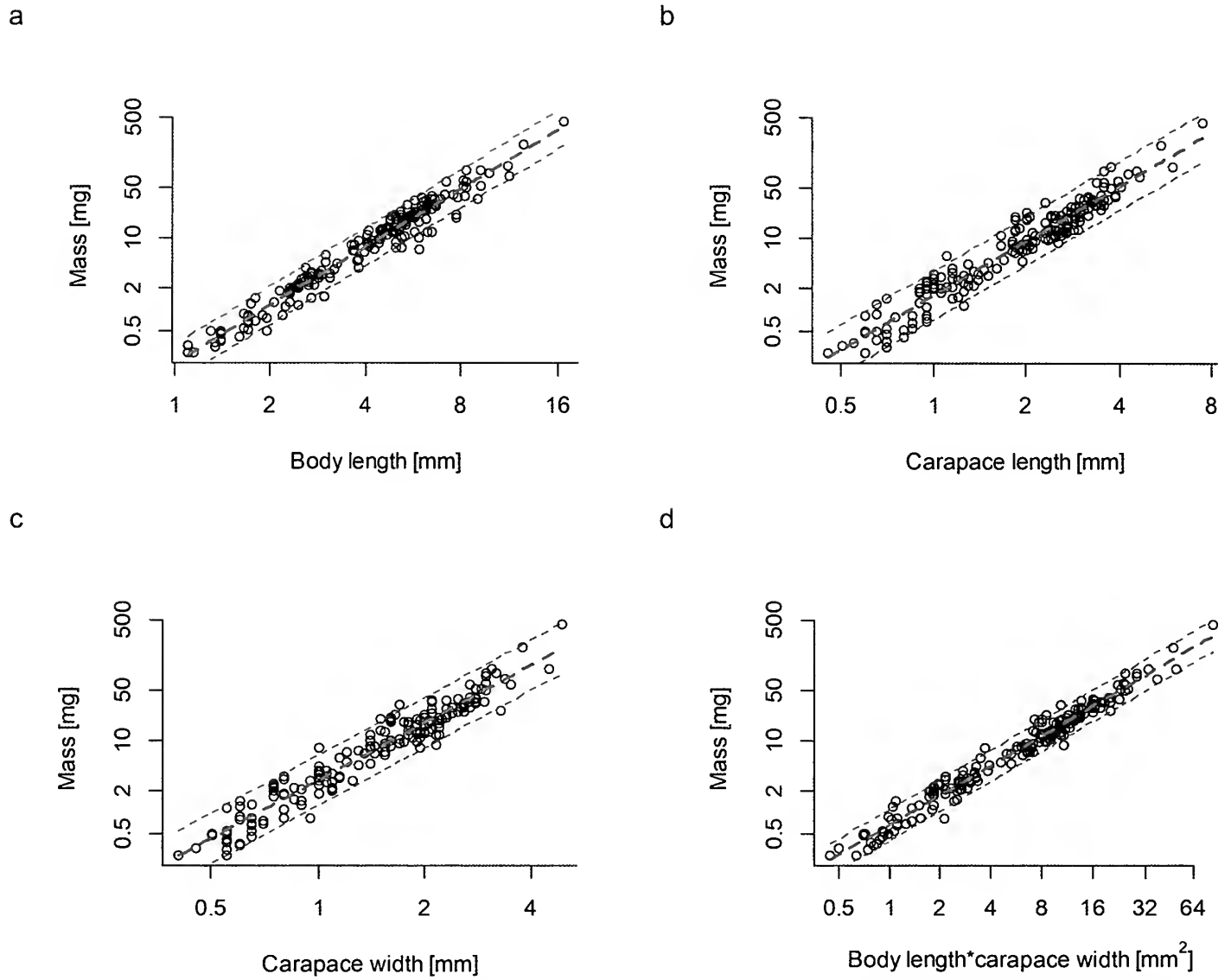

Figure 1.—Scatter plots of spider body size measures/fresh mass and regression lines with prediction intervals (outer lines) calculated for all spiders ($n = 189$): a. Body length/mass. b. Carapace length/mass. c. Carapace width/mass. d. Body length x carapace width/mass.

might reflect the mean biomass of a species better. Of the carapace measures, CL performed slightly better than CW.

Although the use of general equations is desirable and was repeatedly proposed, there is concern that the length-mass relationship might differ between taxa (i.e., with different body shapes), between regions (latitudes, climatic zones, temperate versus tropical faunas; see Schoener 1980; Gowing & Recher 1985; Höfer & Ott 2009; Martin et al. 2014), or

Table 1.—Regression statistics for the different regression equations of the form mass = exp (a + b (ln body measure)): sample size (N), range of body length (BL) in mm, adjusted determination coefficient (adj. R^2), standard deviation of residuals (SD), BL = body length, CL = carapace length, CW = carapace width; all regressions highly significant ($p < 0.0001$).

				BL		CL		CW		BL*CW	
	Group	N	BL range [mm]	adj. R^2	SD	adj. R^2	SD	adj. R^2	SD	adj. R^2	SD
Fresh mass	All spiders	189	1.15 - 16.8	0.959	0.31	0.933	0.39	0.928	0.41	0.972	0.26
	Ground spiders	105	1.10 - 12.6	0.981	0.22	0.950	0.36	0.944	0.37	0.977	0.24
	Vegetation spiders	83	1.10 - 9.0	0.919	0.38	0.908	0.40	0.898	0.42	0.960	0.26
	Males	59	1.15 - 9.2	0.968	0.26	0.953	0.31	0.954	0.31	0.984	0.18
	Females	73	1.30 - 16.8	0.975	0.26	0.944	0.38	0.946	0.38	0.976	0.25
	Lycosidae	58	2.85 - 9.8	0.930	0.17	0.845	0.25	0.806	0.28	0.924	0.18
Dry mass	All spiders	30	1.15 - 8.3	0.911	0.42						

Table 2.—Parameter estimates (a - intercept, b - slope) with standard errors (SE) for the different equations of the form mass = exp (a + b (ln body measure)), BL = body length, CL = carapace length, CW = carapace width.

		BL				CL				CW				BL*CW			
	Group	a	SE	b	SE	a	SE	b	SE	a	SE	b	SE	a	SE	b	SE
Fresh mass	All	-1.72389	0.06	2.69638	0.04	0.47993	0.04	2.51877	0.05	1.04109	0.04	2.66434	0.05	-0.40055	0.04	1.38032	0.02
	Ground	-1.86873	0.06	2.80107	0.04	0.34416	0.05	2.5597	0.06	0.99308	0.05	2.61657	0.06	-0.43006	0.05	1.37422	0.02
	Vegetation	-1.50235	0.11	2.51147	0.08	0.58644	0.06	2.57141	0.09	1.08888	0.05	2.81913	0.11	-0.3910	0.06	1.40548	0.03
	Males	-1.89589	0.10	2.83422	0.07	0.16993	0.06	2.60587	0.08	0.72575	0.05	2.78296	0.08	-0.63171	0.05	1.43728	0.02
	Females	-1.70284	0.08	2.71443	0.05	0.61596	0.07	2.56124	0.07	1.20596	0.05	2.62986	0.07	-0.26268	0.06	1.35597	0.03
	Lycosidae	-1.79359	0.17	2.78452	0.10	-0.1380	0.18	2.9514	0.17	0.7208	0.15	2.8807	0.19	-0.77771	0.15	1.50278	0.06
Dry mass	All	-3.17260	0.23	2.62960	0.15												

between habitat types. Most studies agree that taxon-specific regression equations on the level of orders are necessary (see Hódar 1996; Wardhaugh 2013).

In order to decide which method and equation to choose for the respective scientific question, it would be of particular interest to recognize the reliability and predictive power of general versus specific equations. The applicability of the different regressions for the biomass estimation of spiders has therefore been tested using a test dataset of 166 spiders sampled at different locations and during different seasons of the year to minimize local or seasonal effects.

Lycosids were selected for taxon-specific regressions for several reasons. First, they were sufficiently represented in our samples, due to their abundance and activity. For the same reason, lycosids often (strongly) dominate pitfall trap samples and pitfall trapping is a very common sampling method in ecology. Where wolf spiders are abundant (i.e., in grassland, Jocqué & Alderweireldt 2005), these relatively large spiders dominate the biomass of predators and are supposed to have a considerable impact on both prey and predator assemblages of the ground. So, it seemed reasonable to test a proper regression for this family to increase accuracy. In contrast to our expectation, the estimates using parameters from the taxon-specific regressions differed stronger from the weighed mass than the estimates from general regressions, probably reflecting the lower goodness-of-fit and a resulting lower predictability. The use of habitat specific or sex specific regressions did not produce significantly different biomass estimates. We therefore conclude that a further identification or a separation in male/female or habitat groups is not necessary in order to improve the estimated biomass values.

The taxon-specific (lycosid) regressions showed a lower goodness-of-fit due to the narrow size range and body mass range. Although lower R^2 values are not inherently bad as far as predictions are made for specimens within the same range, estimating the mass of spiders outside of the small range used for regression creation can lead to a considerable bias. It is important to remember that the predictive power comes from the inclusion of many data points distributed over the whole range of size, shape and size-mass relation. A sample size of $n = 10$ can be adequate to find a significant relationship of size-mass with high R^2 values (Hódar 1996), but the probability that the regression is useful for most of the different species/shapes is low, due to low predictive power.

Our sample of 189 specimens including 47 species from 17 families, collected in different habitats and strata represents most of the guilds and life-history types occurring in temperate European habitats, i.e., sheet, space and orb web weavers, ambush, ground and other hunters (Cardoso et al. 2011). Very few Central European spiders fall outside the size range represented by our sample. Based on this and the goodness-of-fit results, we expect our regressions to be useful for spider assemblages at least in temperate regions of Europe.

The slope parameter b (power coefficient) of our regressions based on single body measures ranged between 2.51 and 2.95 for fresh mass (2.63 for dry mass), thus being close to 3 as expected for animals with isometric growth (Suter & Stratton 2011). The values differed only slightly from regressions for Neotropical spiders (Mata Atlântica 2.87, Amazonia 2.98 for all spiders, but 2.2 for Amazonian web-building spiders; Höfer & Ott (2009), Supplementary Material Table 3, online at http://dx.doi.org/10.1636/JoA-S-17-044.s11).

Comparing length-dry mass relationships of assemblages from temperate and tropical zones, Schoener (1980) found that tropical insects usually have smaller power regression coefficients. No difference was found between temperate habitats located far apart from each other (Gowing & Recher 1985). Martin et al. (2014) also showed a positive relation between the absolute latitude of the sample and length-dry mass power regression coefficients. Höfer & Ott (2009) determined parameter estimates from regression analyses for Neotropical arachnids and showed that parameters from different tropical regions within Brazil are close enough to be used, but parameters from temperate zones were not useful for the Neotropical fauna. The slopes of the regressions of Brazilian spiders are higher than the slopes of the regressions of the German spiders. Using the parameters of the linear regression calculated by Höfer & Ott (2009: subtropical Brazil), the relative difference of the estimated to the weighed body mass was 9% higher than using the equation of this study. The use of equations from the tropical region led to 7 - 24% higher differences (Supplementary Material Table 3, online at http://dx.doi.org/10.1636/JoA-S-17-044.s11). Thus, equations from different climatic zones should not be used for mass estimation, while equations from the same zone might be used outside the geographic region.

The ratio between dry and fresh mass measured in this study (0.13–0.34, mean 0.22) was comparable to other studies: Edwards & Gabriel (1998): 0.25–0.39 (mean 0.314), Höfer & Ott (2009): 0.12–0.29 (mean 0.21) in spiders from southern Brazil and 0.24–0.34 (mean 0.29) in spiders from Amazonia. The preservation of spider specimens in 75% ethanol for one

year did not significantly alter body length in this study, although there was a trend towards shrinking. This could become relevant over longer periods of preservation time (Greenstone et al. 1985; Edwards 1996; Simmons 2014), but the bias should not be large and does not matter when comparing specimens sampled and measured at the same time. Anticipating that the soft-skinned opisthosoma is more affected by shrinking or swelling than the strongly chitinized prosoma, regressions based on carapace measures could be applied when using long term conserved material.

Biomass is a good proxy for metabolic rate, food requirement, competitiveness etc., and in the case of spiders as predators also for the impact on prey populations and possibly the carrying capacity (Seidl & Tisdell 1999) of an ecosystem. We therefore suggest our regression parameters as useful for estimating the biomass of spider assemblages in Germany, probably also for the Central European or even Palearctic fauna. Their usefulness for specific questions and precision levels can easily be tested and confirmed, weighing and measuring few specimens from the population, environment or region under study. The slopes of our regressions can also be used to estimate fitness through body condition indices involving size and mass measurements (see Jakob et al. 1996 and following discussion in Kotiaho 1999 and Marshall et al. 1999).

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Survival, abundance, and movement of a synanthropic population of the brown recluse spider, *Loxosceles reclusa* (Araneae: Sicariidae)

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Abstract. We conducted a two-year mark-recapture study of a synanthropic population of the brown recluse spider *Loxosceles reclusa* Gertsch & Mulaik, 1940 in northwestern Illinois. We used joint live encounter and dead recovery models to estimate adult survival, recapture, and dead recovery probabilities. To estimate adult abundance, we used full-likelihood closed-population models. Monthly survival was constant between sexes (0.73, 95% CI = 0.66–0.78), but males were less likely to be recaptured and an additive effect of time revealed highest recapture probabilities in September. The probability of recovering a marked adult that died during the study was 0.13 (95% CI = 0.07–0.24). Average life expectancy for adults was 94 days, much lower than in prior laboratory studies. Causes of observed mortality were predation by conspecifics and cobweb spiders (*Parasteatoda tepidariorum* (C.L. Koch, 1841)) or undetermined. A likely source of mortality for this sit-and-wait predator is starvation. Model averaging of full likelihood closed-population models resulted in adult abundance estimates that were similar between males (28, 95% CI = 20–63) and females (28, 95% CI = 26–38). However, the total population of adults including those hidden in harborage (boxes, furniture, crevices etc.) was undoubtedly much higher. Based on count data, immature spiders were as abundant as adults early in the year, gradually increasing to a peak three times greater by mid-summer. Male spiders moved longer distances than females and were less likely to exhibit site fidelity. The average tenure of a female at a specific site was nearly 8 days.

Keywords: Joint live-dead models, mark-recapture, population structure, recapture probabilities, site fidelity

The brown recluse spider (*Loxosceles reclusa*, Gertsch & Mulaik 1940) is a well-known medically important spider of North America. As such, most prior research has focused on its venom, bite, potential treatments, and distribution (reviewed in Vetter 2015). Early studies of its general biology were conducted largely in the lab; little research has been conducted regarding aspects of its population biology, specifically survival (or longevity), abundance and movements, in more natural settings. Our study aims to fill this gap by following a marked synanthropic population of brown recluse spiders in a semi-natural setting, an urban garage.

Laboratory studies of brown recluse spiders' survival have focused on their apparently unusual longevity. Hite et al. (1966) first reported that female *L. reclusa* lived nearly 3 months longer than males, on average 628 days. Horner & Stewart (1967) housed female spiders individually, kept them at temperatures ranging from 24–36 °C and over-wintered them in a dirt floor of a garage where the low reached -4 °C. Thirty females survived at least four winters or a minimum of 1420 days under such conditions. Female spiders raised by Elzinga (1977) from egg to adult survived an average of nearly 800 days (over 2 years) with one surviving 1755 days or nearly five years. In all of these early studies, spiders were housed individually and fed at least twice a week or even daily. Using a different approach, Eskafi et al. (1977) brought field-captured brown recluse spiders into the lab and modeled the effects of several abiotic factors on weight loss and survival, finding that vapor pressure deficit, temperature, and food stress were the most important factors. Unfed adults lived on average only three months after being brought to the lab. The South American species, *Loxosceles intermedia* Mello-Leitão, 1934, has also been shown to be relatively long-lived in laboratory settings (Fischer & Vasconcellos-Neto 2005), and as in *L. reclusa*, females significantly outlived males.

Studies on *Loxosceles* population size are rarer still and typically consist of reports of minimum estimates based on capture rates in infested buildings, emphasizing the high numbers that may be encountered. Vetter & Barger (2002) reported on a home in eastern Kansas where occupants captured over 2,000 *L. reclusa* in a 6-month period. Researchers in Oklahoma were able to collect over 1,000 spiders from a single barn in three nights of collecting (Vetter 2015). Schenone et al. (1970) found an average of 163 *L. laeta* (Nicolet, 1849) per home in central Chile. Richman (1973) provided one of the few surveys of wild *Loxosceles*, counting *L. arizonica* Gertsch & Mulaik, 1940 by turning over downed saguaro cacti and other plant litter in a desert habitat. He observed that abundance declined in summer while the percent of spiderlings (< 3 mm) increased in winter. About 50% of the total spiders observed were immature.

Ferreira et al. (2005) reported on both abundance and movements of the cave-dwelling *L. similis* Moenkhaus, 1898, in Brazil. Using batch marking methods, they estimated population size using a modified Lincoln-Petersen index. Numbers fluctuated from 800 to over 1400 individuals in the main corridor of the cave which was 140 m long. Estimating individual movement, they found that some spiders moved 10–80 m in a single week. In contrast, Fischer (1996) reported much more limited movements, a mere 2 m/wk, in *L. intermedia*. The general consensus of arachnologists that *Loxosceles* are not great dispersers (Vetter 2015) is supported by studies revealing a largely sedentary behavior (Cramer 2015).

Here we present a demographic analysis of a population of brown recluse spiders inhabiting a large urban garage. Our study is the first to use individually marked adult brown recluse spiders to shed light on survival, abundance, and movements in a non-laboratory setting.

METHODS

We marked 99 brown recluse spiders with unique tags in a large (20 × 10 m) urban garage in Monmouth, IL (USA) during 22 marking periods between June and September 2014. We created individual paper tags approximately 1.5 mm^2 with a unique two-digit or two-letter code in size three font on card stock. To avoid blurring of the code, we applied clear artist's spray fixative before cutting and trimming the tags to size. We then captured adult spiders, anesthetized them using carbon dioxide and affixed the tags to the cephalothorax with SuperGlue™. We used the blunt tip of a paper clip to apply a small drop of glue to the cephalothorax and, using fine forceps, immediately lowered a tag onto the glue. Behavioral and survival effects of carbon dioxide anesthesia have been reported in arthropods (Nicolas & Sillans 1989) and we cannot completely exclude this as having an impact on our study. However, our spiders were fully immobilized for approximately one minute after which they recovered rapidly and were released. Sixty-five percent of marked spiders were recaptured at a subsequent date and showed no apparent ill effects. One marked female successfully overwintered and produced an egg sac that hatched 74 offspring.

We surveyed spiders under red light over two active seasons (mid-May to mid-October) from 2014–2015 by searching the garage for approximately 1 hour between 22:00 and 24:00. Following a prescribed path roughly along the inner perimeter of the garage (but also investigating near undisturbed areas such as stored vehicles and other items), we searched every 2–4 days for marked and unmarked spiders, using a handheld magnifier when necessary to read marks. Thus, in this study a "recapture" indicates a spider that was seen again but not physically captured and released. In all, we sampled 100 times over the two-year study. Additionally, we divided the garage into a grid of 0.5 m^2 sections and recorded the location of each marked spider when encountered.

Population modeling.—We used mark–recapture models within program Mark version 8.2 to estimate population parameters (White & Burnham 1999) and to model survival and abundance. For model selection, we used an information-theoretic approach and Akaike's information criterion adjusted for small sample size (AICc; Akaike 1973; Burnham & Anderson 2002). We used model averaging and unconditional standard errors to account for model selection uncertainty if the top ranked model received < 90% of the model weight (Anderson 2008).

Survival.—To estimate adult survival, we used joint live encounter and dead recovery models (Burnham 1993; Williams et al. 2002). These models use conditional likelihood to estimate survival (S), recapture (p), dead recovery (r), and fidelity (F) probabilities. Emigration is assumed to be a random process (Burnham 1993). Briefly, live animal encounters are recorded at each sampling occasion followed by an interval where recovered dead animals are recorded (Williams et al. 2002). This sampling design results in construction of a paired live/dead encounter history where animals are recorded as encountered alive (1) or unobserved (0) during the live sampling occasion and as dead (1) or unobserved (0) during the interval following the live sampling occasion. Excluding immatures and spiderlings, which were too sparse to model, resulted in a truncated encounter history of 80 paired live encounter and dead recovery events. We adjusted intervals lengths to correct for uneven sampling and estimate monthly (30-day) adult survival. Because all live encounters (with two exceptions, see results for details) and dead recoveries occurred within the sampling area, we fixed the fidelity parameter to 1 in all models (Cooch & White 2017).

Our global model of survival (S) included sex as a factor and season as an additive effect. We modeled season as a dummy variable to evaluate if monthly survival during the active season (6 June–7 Oct 2014 and 13 May–27 Sept 2015) differed from monthly survival during quasi-winter, defined here by the sampling occasions that bracketed winter (7 Oct 2014–13 May 2015). The recapture parameter (p) included a sex by time interaction. The dead recovery parameter was treated as time invariant but with differing probabilities based on sex. We assessed the fit of the global model by estimating an overdispersion factor ($\hat{C}$, Fletcher 2012). If overdispersion was detected (i.e., $\hat{C} > 1.00$), we assumed it was due to parameter heterogeneity and corrected for it using quasi-likelihood and $QAIC_c$ (Wedderburn 1974; Burnham & Anderson 2002). We include 13 nested iterations of the global model for a total of 14 candidate models (Table 1). We estimated adult life expectancy (e_x) assuming constant survival using life table analysis and the following life expectancy equation: $e_x = \frac{T_x}{l_x}$, where (T_x) is the number of monthly intervals survived beyond the first month as an adult, and (l_x) is the fraction of adults alive at beginning of the first month interval (Case 2000).

Abundance.—To approximate the assumption of demographic closure (i.e., no births, deaths, immigration or emigration) so that closed-population models could be used to estimate abundance, we identified and excluded immature captures. In addition, we restricted analysis to adults captured in 2014 during a period of ≤ 19 days for each sex to maximize the number of captures yet reasonably assume no mortality during the sampling period.

We estimated sex-specific adult abundance using full likelihood closed-population finite mixtures models (Norris & Pollock 1995; Pledger 2000). These models estimate four fundamental parameters: probability of first capture (p), probability of recapture (c), number of animals never captured (f), and a finite mixture (π). The number of animals never captured (f) forms part of the likelihood and is required to derive estimates of abundance but is not explicitly modeled. The remaining three parameters are modeled and fitted to the data (Chao & Huggins 2005). We considered two finite mixtures to account for latent heterogeneity in capture and recapture probabilities and fixed mixtures to one for models that did not include latent heterogeneity effects so that the eight models of Otis et al. (1978) could be compared using the same likelihood-framework (Pledger 2000; Cooch & White 2017). These models range in complexity from a null model that treated capture and recapture probabilities as constant, to the most parameterized model that included time (t), behavior (b, a response induced by initial capture and marking), and latent heterogeneity effects (2 mixtures) to explain capture and recapture probabilities (Otis et al. 1978; Chao & Huggins 2005). Including sex effects among the parameters described above resulted in 16 candidate models. We used R version

Table 1.—Fourteen joint live encounter and dead recover candidate models used to estimate adult monthly survival. Model parameters include survival (S), recapture (p), and dead recovery (r) probabilities. Sex is a factor with two levels. Time is considered alone or as an additive (+) or an interaction (*) effect with sex. Season (a constrained time model) is a factor with two levels: active season and quasi-winter. The notation (.) indicates the parameter is treated as constant.

Model	$QAIC_c$	$\Delta QAIC_c$	w_i	K	-2log(£)
S (.) p (sex + time) r (.)	-10993.030	0.000	0.947	79	1597.153
S (season) p (time) r (.)	-10986.944	6.086	0.045	79	1603.681
S (.) p (time) r (sex)	-10982.704	10.326	0.005	79	1608.229
S (sex) p (time) r (.)	-10981.277	11.753	0.003	79	1609.759
S (season) p (sex + time) r (.)	-4836.807	6156.223	0.000	80	1590.956
S (sex) p (sex + time) r (.)	-4831.180	6161.850	0.000	80	1596.991
S (sex + season) p (sex + time) r (sex)	-1762.323	9230.707	0.000	82	1583.966
S (sex + season) p (sex * time) r (sex)	1092.243	12085.273	0.000	157	1508.414
S (season) p (sex) r (.)	1563.751	12556.781	0.000	5	1665.758
S (.) p (sex) r (sex)	1567.812	12560.842	0.000	4	1672.561
S (.) p (sex) r (.)	1567.833	12560.863	0.000	5	1670.137
S (sex) p (sex) r (.)	1570.074	12563.104	0.000	5	1672.541
S (.) p (.) r (.)	1586.178	12579.208	0.000	3	1694.644
S (.) p (time) r (.)	1657.525	12650.555	0.000	78	1610.620

Models are ranked in ascending $QAIC_c$ order. $\Delta QAIC_c$ is the $QAIC_c$ difference between model i and the top-ranked model. w_i is the adjusted model weight, K is the number of parameters, and -2log(£) is a measure of the relative fit for a given model.

3.4.0 (R Core Team 2017) and the ggplot2 package (Wickham 2009) to graphically depict recapture probabilities.

Population structure.—We surveyed marked and unmarked spiders, stratifying individuals as adult males, adult females, immatures ($\geq$ 3 mm body length) and spiderlings ($<$ 3 mm), and tallied count data for each grouping. We restricted our analysis of population structure to the 2015 sampling season (13 May–27 Oct) because temporal sampling across age classes was sporadic in 2014. To aid visual representation of seasonal changes in population structure, we pooled sexes for adults and used a stacked bar graph created in IBM SPSS Statistics 21 (SPSS Inc., Chicago, IL, USA).

Movement and site fidelity.—To estimate movement frequency and distance, we recorded the interval (days) between sightings and estimated the minimum linear distance traveled between point locations on those dates. We also estimated site fidelity to a given location using an *a priori* definition of "resident" spider as one moving less than 1 m between observations and encountered at least twice after its initial marking but with no more than one week between encounters. The rationale for the latter restriction was to adequately approximate the assumption that a spider had not left the site for a significant period of time and later returned. These data were analyzed on Minitab (Minitab Inc., State College, PA, USA) using nonparametric tests (Spearman's correlation, Mann-Whitney). For frequentist statistics, we *a priori* set $\alpha = 0.05$.

RESULTS

We encountered 99 spiders (79 adults and 20 immature individuals) 385 times and recovered 11 adult dead spiders (7 males, 4, females) between 6 June–7 Oct 2014 and 13 May–27 Sept 2015. Sixty-five percent were seen on at least one subsequent occasion and we observed one tagged female that successfully overwintered and produced and guarded an egg sac that hatched 74 spiderlings the spring following her first capture. One dead, recovered adult male likely died due to complications from a forceps injury. This individual was treated as a known removal (-1) in the joint live encounter and dead recovery analysis, along with two other adult marked males discovered well outside the survey area, one of which may have been inadvertently transported when an item was removed from the garage.

Survival.—We detected slight evidence of overdispersion ($\hat{C} = 1.07$) for the global model so we used $QAIC_c$ for model selection and inflated our variance estimates accordingly using the overdispersion factor ($\hat{C}$). The top-ranked model garnered 0.947 of the model weight (w_i, Table 1). The majority of the remaining trivial support ($w_i = 0.045$) went to a model where survival varied based on season, recapture probabilities varied by time, and dead recoveries were treated as constant. Thus, model averaging was unnecessary. The strongly supported top-

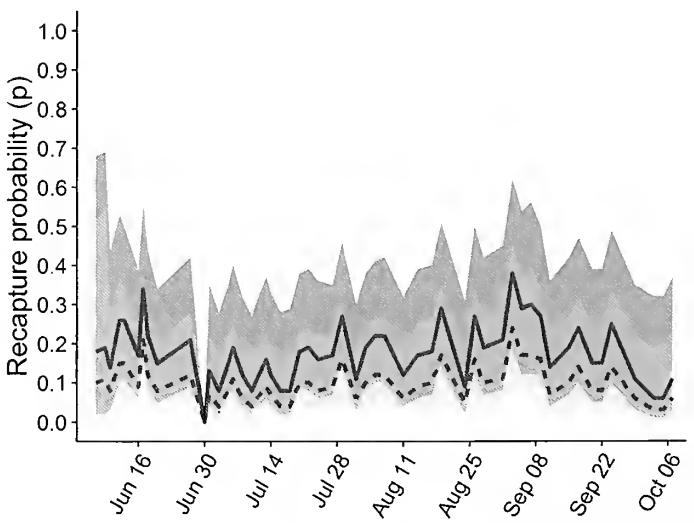

Figure 1.—Day of year (2014) plotted against sex specific adult recapture probabilities resulting from the joint live encounter dead recovery analysis. Solid black lines represent female point estimates and 95% CI (dark gray). Dashed black lines represent male point estimates and 95% CI (light gray).

Table 2.—Sixteen full likelihood closed-population candidate models used to estimate adult abundance. Models vary in their treatment of sex, time (M_t), behavior (M_b), and heterogeneity (M_h) to explain capture and recapture probabilities. Interactions among parameters are denoted by (*).

Model	AIC_c	ΔAIC_c	w_i	K	-2log(ℒ)
Sex*M_0	203.729	0.000	0.717	4	194.704
Sex*M_b	206.331	2.602	0.195	6	192.061
M_0	210.240	6.510	0.028	3	203.640
M_b	210.424	6.695	0.025	4	201.399
M_{bh2}	211.563	7.834	0.014	6	197.292
M_{h2}	211.708	7.979	0.013	5	200.129
Sex*M_{h2}	213.161	9.432	0.006	8	193.047
Sex*M_{bh2}	216.451	12.722	0.001	10	189.784
M_t	227.268	23.539	0.000	11	197.018
M_{tb}	230.554	26.825	0.000	12	196.490
M_{th2}	231.425	27.696	0.000	13	193.292
M_{tbh2}	234.701	30.972	0.000	14	192.218
Sex*M_t	253.484	49.755	0.000	20	176.962
Sex*M_{tb}	268.155	64.426	0.000	22	175.964
Sex*M_{th2}	285.951	82.222	0.000	24	174.793
Sex*M_{tbh2}	307.523	103.794	0.000	26	172.935

Models are ranked in ascending $QAIC_c$ order. $\Delta QAIC_c$ is the $QAIC_c$ difference between model i and the top-ranked model. w_i is the adjusted model weight, K is the number of parameters, and -2log(ℒ) is a measure of the relative fit for a given model.

ranked model treated monthly survival as constant between sexes but allowed recapture probabilities to vary by sex (males were less likely to be recaptured) with an additive effect of time (Fig. 1, only recapture probabilities from 2014 shown). Mean 30-day adult survival probability was 0.73 (95% CI = 0.66–0.78). Average life expectancy for adults was 94 days.

The probability of recovering a marked adult if it died during the course of the study was constant between sexes (0.13, 95% CI = 0.07–0.24). Recapture probabilities were on average highest in September followed by June, August, July and October (Fig. 1). From 2014–2015, recapture probabilities ranged from 0.00–0.56 for males and 0.00–0.72 for females, but female recapture probabilities (0.23) were higher than those of males (0.13) on average.

Abundance.—Of the 16 full likelihood closed-population models considered, only the top-ranked model that treated captures and recaptures as equal but differing by sex received notable support (w_i = 0.717, Table 2). The next most supported model (w_i = 0.195) was an embellishment of the model above but included a behavioral effect which treated captures and recaptures as unequal. However, this effect was considered uninformative as it did not decrease the -2log likelihood enough (≥4 units) to overcome the AIC_c penalty term for having two more parameters than the top-ranked model. The remaining models received little ($w_i \leq 0.028$) to no support. Model averaging of full likelihood closed-population models resulted in abundance estimates of 56 adults with a 1:1 sex ratio for 2014 (Table 3). The density of adults in this 200 m^2 garage was approximately 0.28 spiders/m^2 although spiders were not evenly dispersed but clustered near walls and other potential refugia such as permanently stored vehicles and furniture.

Table 3.—Model averaged estimates of adult abundance from candidate models in Table 2. M_{t+1} = number of unique individuals encountered; $\hat{N}$ = abundance estimate.

Group	M_{t+1}	$\hat{N}$	SE	Lognormal 95 % CI
Males	18	28	8.9	20 – 63
Females	26	28	2.4	26 – 38
Combined	44	56	9.5	47 – 91

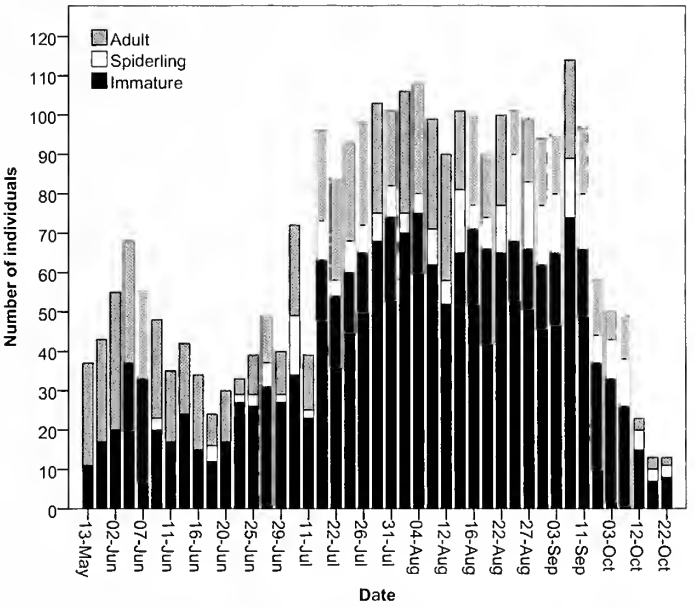

Figure 2.—Stacked bar graph representing the total number of unique adults (gray), immatures (black), and spiderlings (white) observed on a given date in 2015.

Population structure.—Summed over 13 May–27 Oct 2015, we had 241 observations of adult males, 563 observations of adult females, 1,828 observations of immatures, and 286 observations of spiderlings. Because of their small size, spiderlings may be underrepresented in our counts. For a given day, the number of observations was equal to the number of unique individuals observed (Fig. 2). The first detection of spiderlings occurred on 9 June (Fig. 2), less than one month after spiders became active. An approximate three-fold increase in immatures observed began 10 July and persisted through 11 September.

Movement and site fidelity.—As noted above, females were recaptured more often than males. Distance moved (m) between recaptures was positively correlated with interval (days) between captures (Spearman $\rho = 0.57$, $P < 0.001$). Males moved five times farther on average than females (Fig. 3), with a median ($\tilde{X}$) of 0.6 m/day versus 0.12 m/day for females (Mann-Whitney test; $P < 0.001$; 95% $\tilde{X}_F - \tilde{X}_M = -0.37$ [CI ± – 0.17]). Two males traveled to an adjacent building; one that traveled 20 m was excluded from this analysis because there was a remote chance it may have been transported accidentally when the homeowners moved some items from the garage to the house. Excluding the second male (that moved 28 m) discovered in the adjacent house did not significantly change the results or estimates (Mann-Whitney test; $P < 0.001$; $\tilde{X}_F - \tilde{X}_M = -0.33$ [CI +/- –0.14]). No females were observed to leave the garage; the maximum distance traveled by a female was 20.5 m.

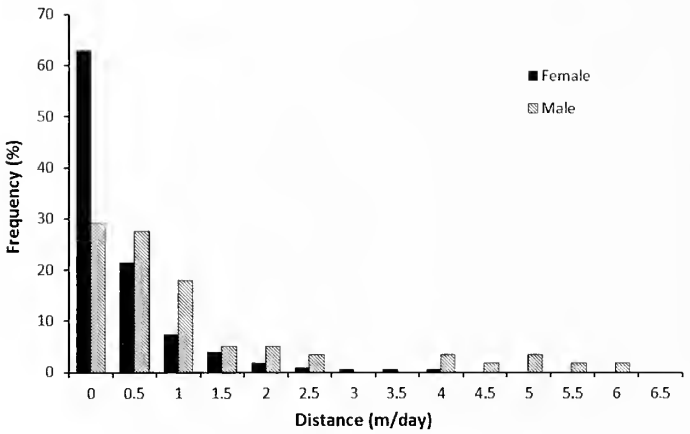

Figure 3.—Frequency distribution of mean distance moved per day by male (n = 61) and female spiders (n = 228). Two outliers, one female at 10.5 m/day and one male at 12 m/day are not shown.

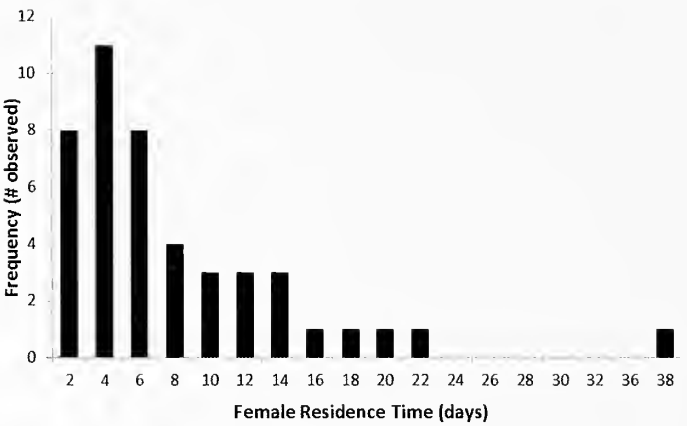

Figure 4.—Frequency distribution of the number of days a resident female spider (n = 45) remained in the same 1 m^2 area. Spiders were considered resident if they were encountered at least twice after marking within 1 m or less of their previous location (with no interval between encounters greater than one week). Males are not shown because only 4 males met the definition of resident.

Mean distance moved per day can be misleading given that spiders would often stay in the same area for a few days and then move a long distance in a short period of time. For this reason, we established a definition of a resident spider in order to analyze the site fidelity of those resident spiders. We found 34 instances (77%) in which females could be defined as resident, but only four males (19%) that met our definition (X^2 = 19.8, $P < 0.001$). The mean tenure for resident females was 7.7 days (SE = 1.01) with an extreme of 38 days (Fig. 4). A female observed guarding an egg sac for 28 days was excluded from this analysis. While the sample is too small to statistically compare mean differences between the sexes, males (n = 4) stayed on average 4.3 days (SE 1.65) at a site.

DISCUSSION

Observed longevity in *Loxosceles* from laboratory studies (Hite et al. 1966; Horner & Stewart 1967; Elzinga 1977; Eskafi et al. 1977) is dramatically higher (up to four years) than our estimate of life expectancy for adults (94 days or 1.3 years from hatching) in a free-ranging synanthropic population. Other mark-recapture studies demonstrate the wide variability among spider species in survival and recapture rates. Framenau & Elgar (2005) marked a population of wolf spiders using bee tags and found a very high rate of survival from 75-85% over 6 months. Male survival was lower in the spring cohort likely because of risks associated with searching for mates. In contrast, survival in our population was 73% per month (not six months) and we found no difference in male and female survival rates. Framenau & Elgar (2005) also report substantially higher recapture probabilities (0.3–0.7) than ours (Fig. 1). Interestingly, they report lower recapture rates for females than males, the opposite of our results. In another study of a smaller species of wolf spider, *Pardosa agrestis* (Westring, 1862), Kiss & Samu (2000) reported recapture rates comparable to ours (0.19 to 0.5) in alfalfa fields.

Survival rates in *Loxosceles reclusa* could be influenced by predation mortality from other spiders, including conspecifics. Of the 11 instances of mortality we observed, two (one male, one female) were caught in webs of *Parasteatoda tepidariorum* (C.L. Koch, 1841). Sandidge (2004) also noted predation by this common house spider on brown recluse spiders, observing a weak negative correlation between the presence of *P. tepidariorum* and *Loxosceles* in synanthropic populations. Cannibalism is widespread in spiders (Wise 2006) and not uncommon in *Loxosceles*. Cramer (2015) observed that spiders comprised 25% of prey captured by *L. reclusa*, and 40% of those were conspecifics, thus comprising 10% of their diet. In the current study, 3 of the 7 males recovered dead were cannibalized by females. Together, these observations suggest that cannibalism is potentially a significant source of mortality in *L. reclusa*.

Despite their low metabolism (Carrel & Heathcote 1976) and longevity in the lab, starvation is probably a significant cause of mortality in *L. reclusa* in natural populations. Six of the eleven marked spiders that we found dead in this study had no evidence of other causes of death. Eskafi et al. (1977) found that temperature and vapor pressure deficit were significant factors in brown recluse spider survival, but that spiders which had fed before beginning the experiment endured significantly longer. Brown recluse spiders have very low rates of water loss and rely on metabolic water when water stressed, but lack of food ultimately caused death within 55 to 94 days. In contrast, spiders that began the experiment "engorged" increased their survival to 151 to 222 days. Our calculated life expectancy of 94 days is in the upper range of spiders who have not fed, lending credence to the argument that starvation is a biologically important source of mortality. Although Sandidge (2003) considered scavenging to be a significant source of nutrition for brown recluse spiders that enabled them to persist in urban settings and avoid starvation, its importance has been contested in subsequent studies (Cramer 2008, 2015; Vetter 2011). Observations by Cramer (2015) demonstrated that *L. reclusa* is a sit-and-wait predator that does not actively search for prey and therefore is unlikely to scavenge even when freshly dead prey items are placed within a meter of their webs.

Accurate estimates of *Loxosceles* abundance or density that include some estimates of error are lacking. Most are simply reports of enormous numbers (usually *L. reclusa*) captured in

short periods of time in various structures (e.g., Schenone et al. 1970; Vetter & Barger 2002). Thus, our abundance estimate of 56 adults (05% CI 47–91) or 0.28 adult spiders/m^2 are not comparable to these studies. Including immature spiders elevates our density estimate to one spider/m^2 or 200 spiders in the entire structure. However, our estimate is likely low because many areas of the garage could not be inspected due to, e.g., abundant harborage in the floor, walls, and stored materials, as well as the rafters and ceiling. Our estimate is limited to spiders principally on the floor or visible without disturbing materials stored in the garage.

Other mark-recapture studies show that site fidelity varies among species of arachnids. Samu et al. (1996) created artificial web sites for linyphiids inhabiting wheat fields in order to examine site fidelity. Spiders frequently moved webs, averaging only 1.7 days per site with a maximum tenure of 8 days. Such shifts were caused equally by competition from conspecifics, destruction of a web, or simple abandonment. In contrast, the wolf spider *Pardosa agrestis* adopted a "sit-and-move" foraging strategy (Samu et al. 2003). Ninety percent of the time, the spiders sat inactive (for periods of 2.5 min on average). These periods of inactivity were interrupted by brief periods of movement to change location. Both sexes moved on average < 4 m/day. Similar to our results, movement distance was highly skewed toward shorter distances, though some individuals moved up to 20 m/d. Arguably, brown recluse spiders also adopt a sit-and-move strategy, but with much longer intervals between significant movements, on the order of days rather than minutes. In another study of wolf spiders, Ahrens & Kraus (2006) found that no more than half of the individuals of the riparian spiders (*Pirata* spp.) they studied moved to an adjacent one meter section per day; many never moved even one meter.

Other researchers have reported significant sex differences in movement and site fidelity as we report here for brown recluse spiders. Dodson et al. (2015) studied site fidelity and movements in another sit-and-wait (or sit-and-move) predator, *Misumenoides formosipes* (Walckenaer, 1837). Using a variety of methodologies, they found that males moved six to ten times farther than females. Females also remained at a site more than twice as long as males. This is consistent with many studies of male and female behavioral differences in arachnids where males are more mobile, presumably in a search for mates, which potentially exposes them to greater risks of mortality. For instance, Kasumovic et al. (2006) showed that mate searching in *Nephila plumipes* (Latreille, 1804) exposed males to high mortality risk (a survival rate of 36% over the 25-day course of their mark-recapture study). Even this tiny male orb weaver would travel about an average of 10 m in 3.5 days. Hebets (2002) also demonstrated sex differences in site fidelity in amblypygids. While there was no difference in frequency or distance of movement, females were much more likely to be sedentary. Framenau (2005) recorded greater activity of male than female wolf spiders, but only during two months when males were searching for more sedentary females. As in our study, males were less often recaptured than females, most often being encountered only once after marking. Framenau (2005) suggested that selection for longer leg length (sex dimorphism) in males was driven by mate searching behavior. *Loxosceles reclusa* is also a sexually dimorphic species in regard to relative leg length. Measurements of *L. reclusa* taken by Gertsch & Ennik (1983) show that all male legs are longer than females, especially the first two pair, by 19 and 33%, respectively. Nonetheless, while male brown recluse spiders in our study moved more frequently and farther than females, we did not detect any difference in overall survival between the sexes.

Movement and site fidelity may be linked to mortality risk from starvation which is a widespread in spiders (Wise 2006). Variation in site tenure in brown recluse spiders may reflect a "win/stay – lose/shift" strategy as seen in pholcids that was related to food availability and presence of conspecific competitors (Jakob 2004). She noted that spiders would leave the web when food-deprived and would be more likely to do so if a larger conspecific were sharing the web. Similarly, Miyashita (2005) reported that giving supplemental food to *Nephila* increased residence time. Crab spiders, *Mecaphesa* (as *Misumenops*) *asperatus* (Hentz, 1847) prefer patches of goldenrod with a greater number of inflorescences and spiders in higher quality patches remain there longer (Robakiewicz & Daigle 2004). In contrast, Vetter & Rust (2008) found no link between degree of starvation and tendency to shift refugia in a laboratory study of *L. reclusa*. On average, spiders shifted refugia every two to three days, about twice as often as we observed. Gillespie & Caraco (1987) reported a somewhat counterintuitive strategy in long-jawed spiders. Low prey availability led to increased residence time whereas in areas of high prey availability spiders were more likely to relocate. Although we did not directly estimate prey availability, this hypothesis suggests another avenue to investigate to explain the sedentary, sit-and-wait foraging strategy of brown recluse spiders.

In this paper, we examined survival, abundance and movement of *L. reclusa* as well as differences in these variables between the sexes. We report an average life expectancy of 94 days for adult spiders, far less than reported for spiders reared in the laboratory, some of which have lived for more than four years. Thus, turnover in synanthropic populations of brown recluse spiders is much higher than what can be inferred from prior laboratory studies. The abundance of adult spiders we report in this setting is consistent with prior anecdotal reports but perhaps underestimated due to an inability to detect all individuals by thoroughly searching the entire structure. Females were far more sedentary than males, often remaining in the same area for more than a week. Males moved much more frequently than females, traveled greater distances and were less likely to be recaptured. Nonetheless, despite this marked difference in movements, we detected no difference in survival between males and females.

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Habitat associations of the web-building wolf spiders *Sosippus floridanus* and *Sosippus placidus* (Lycosidae: Sosippinae): a widespread generalist versus an endemic specialist

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Abstract. Habitat associations of two species of congeneric web-building wolf spiders were examined in their zone of syntopy on the Lake Wales Ridge in Florida. The species differed in use of all of the habitat variables measured. The geographically widespread species, *Sosippus floridanus* Simon, 1898, associated with habitat features typical of scrubby flatwoods and human disturbed areas, which are found throughout the Florida peninsula. The endemic species, *S. placidus* Brady, 1972, associated with a scrub oak habitat that is restricted to the Lake Wales Ridge. Biogeographic implications of these differences are discussed.

Keywords: Florida scrub, biogeography, web-site selection, syntopic

Lycosidae is a large family of spiders (2421 species, 124 genera) distributed world-wide in a great range of habitats (World Spider Catalog 2018). The vast majority are vagrant hunters, either lying in wait for or wandering in search of prey. Some species are sedentary, building more-or-less permanent burrows from which they ambush passing prey, and only a minority of species build prey capture webs (Murphy et al. 2006). Wolf spiders in the subfamily Sosippinae are all obligate web-builders (Brady 2007). The genus *Sosippus* Simon, 1888 builds webs that exhibit a remarkable resemblance to webs of the funnel-web building family Agelenidae. In fact, there was some intitial speculation that *Sosippus* might represent a transitional genus between the Agelenidae and Lycosidae (Brady 1972), however more recent analysis does not support that position (Griswold 1993; Griswold et al. 1999). The current view is that while it is not monophyletic, sheet-web building is an ancestral trait in lycosids, which once lost is unlikely to have been regained (Murphy et al. 2006).

The geographic distribution of *Sosippus* ranges from the southern United States, through Mexico and Central America to Costa Rica (Brady 1962, 2007). There are currently ten described species. The only species that have been studied in any depth are the four that occur on the Florida Peninsula (Brady 1972, 2007). The geographic distribution of Florida *Sosippus* is consistent with an evolutionary history of relict southeastern expansion during glacial periods, with speciation and isolation on islands during interglacial periods (Marshall et al. 2000). Of interest to our study is the isolation of areas of habitat defined as Florida scrub. During interglacial periods when sea levels rose, the higher areas of Florida became isolated as a series of islands (Deyrup 1989; Myers 1990; Webb 1990). These islands accumulated great depths of sand around their margins. Isolated from one another these islands each evolved unique flora and fauna. After sea level dropped, these deep piles of sand were scattered down the central area of the Florida peninsula. Even though they currently receive the same abundant rainfall typical of Florida in general, they retain their dry adapted plants and animals because any rainfall they receive rapidly drains from the deep sandy soils. Thus, each of these ancient islands possess plants and animals found nowhere else in the world (Deyrup 1989; Marshall et al. 2000).

The distribution of *Sosippus placidus* Brady, 1972 is restricted to one of these habitat islands, the Lake Wales Ridge in south central Florida (Brady 1972, 2007). Its distribution is so restricted, primarily to Archbold Biological Station (ABS) and a few scrub remnants near Lake Placid, Florida (hence, the species name), that it was listed as endangered by the Florida Committee on Rare and Endangered Plants and Animals (Edwards 1994). In contrast, *Sosippus floridanus* Simon, 1898 co-occurs with *S. placidus* at ABS but also extends from southern Georgia, through the Florida peninsula to the Florida Keys (Brady 1972, 2007). Brady (1972, 2007) has suggested that the historical Pleistocene isolation led to subsequent reduction in genetic variability, limited dispersal abilities and ecological specialization for xeric conditions by *S. placidus*. The widespread distribution of *S. floridanus* may be due to originally having had a more northern distribution, escaping isolation on the xeric Pleistocene islands, and then spreading down the Florida peninsula after the interglacial. In this study, we examine these hypotheses by comparing web site characteristics of these two species at ABS, where they are syntopic. We predicted that *S. placidus* would show scrub-specific habitat associations whereas *S. floridanus* would exhibit a more generalized set of web site characteristics reflecting a lack of ecological specialization.

METHODS

Research was conducted at Archbold Biological Station, an independent ecological research facility located in Venus, Florida, between May–June 2002. Visual search was used to locate webs which are visible in the early morning when covered with dew. The field station has an extensive network of fire roads which were used to access the scrub. A total of ten different surveys (on ten different days) were conducted. When a web was located, a meter stick was positioned above it, parallel to the direction of the funnel and centered on it at the 50-cm mark. The species was identified by flushing the spider out of the funnel onto the web. The two species are visually

Table 1.—Standardized discriminant function coefficients predicting the placement of *S. placidus* webs ($n = 149$) versus *S. floridanus* webs ($n = 112$) ranked using *F* scores. Due to lack of normality, the significance level is based on Mann-Whitney U tests. Pooled within-groups correlations between discriminating variable and standardized canonical discriminant functions show that only grass (negative value) predicts *S. floridanus* websites whereas all other variables (positive values) predict *S. placidus* websites.

Habitat variable	*F*	*P*	Canonical correlation coefficient
Pine litter	124.07	<0.0001	0.594
Grass	79.80	<0.0001	-0.476
Cactus	31.58	0.0005	0.192
Sand	25.76	<0.0001	0.271
Leaf litter	24.85	<0.0001	0.266
Gopher apple	18.60	<0.0001	0.230
Vine	16.90	<0.0001	0.219
Lichen	14.61	<0.0001	0.204

quite distinct from each other (*S. floridanus*: carapace with distinct white median stripe, legs brownish-yellow; *S. placidus*: carapace with broad marginal stripes of pale orange, legs with alternating light and dark stripes; Brady 2007). We used a line-intercept method to record habitat variables (Riechert 1976; Marshall & Martin 2011). The presence of plant species and substrate characteristics was recorded along the meter stick at 10 cm intervals. Substrate features included deciduous leaf litter, pine litter, and no litter (open sand). Vegetation included grass (species undetermined), cactus (*Opuntia humifusa*), gopher apple (*Licania michauxii*), vines (*Smilax auriculata* and *Vitis rotundifolia*) and lichen (*Cladonia* spp.). Once a web was scored, it was marked with a flag to avoid re-census. Two sets of transects were conducted. In one set the characteristics of *S. placidus* webs ($n = 149$) were compared to those of *S. floridanus* ($n = 112$). To determine if any particular feature of scrub characterized *S. placidus* webs, a separate set of transects compared a web transect ($n = 39$) to an additional random transect ($n = 39$) that was taken a regular distance away. This random transect was chosen by taking a number of steps directly away from the end of the transect as determined by pairs of numbers from a random number table. The first number was the number of steps away, and the second number then determined a number of steps to the right (if the digit was even) or to the left (if the digit was odd) of the first endpoint. Discriminant analysis was used to determine habitat association (JMP ver. 13, SAS Institute Inc.) using site (*S. placidus* web, *S. floridanus* web, or random site) and habitat variable as factors. Since none of the variables were normally distributed, univariate non-parametric tests (Mann-Whitney U) were conducted on each variable rather than *F* statistics (Riechert 1976; Marshall 1997). Voucher specimens have been deposited at the Denver Museum of Nature and Science.

RESULTS

Comparison of *S. placidus* and *S. floridanus* websites.—A total of 149 one meter transects centered on *S. placidus* websites and 112 centered on *S. floridanus* websites were completed. There were clear differences in the websites of *S. placidus* and *S. floridanus* with respect to all of the habitat variables measured (Table 1, Fig. 1). Of all of the variables tested, discriminant analysis shows that only grass was strongly associated with *S. floridanus* websites, whereas pine litter and open sand were strongly associated with *S. placidus* websites (Table 1). We compared the average number of 10 cm transect intervals occupied by each habitat variable on the 1m transects through *S. floridanus* ($n = 112$) and *S. placidus* webs ($n = 149$). We found that *S. floridanus* was primarily associated with grass and *S. placidus* was primarily associated with pine litter (Fig. 1).

Comparison of *S. placidus* websites and random sites.—Comparing transects through *S. placidus* webs ($n = 39$) with

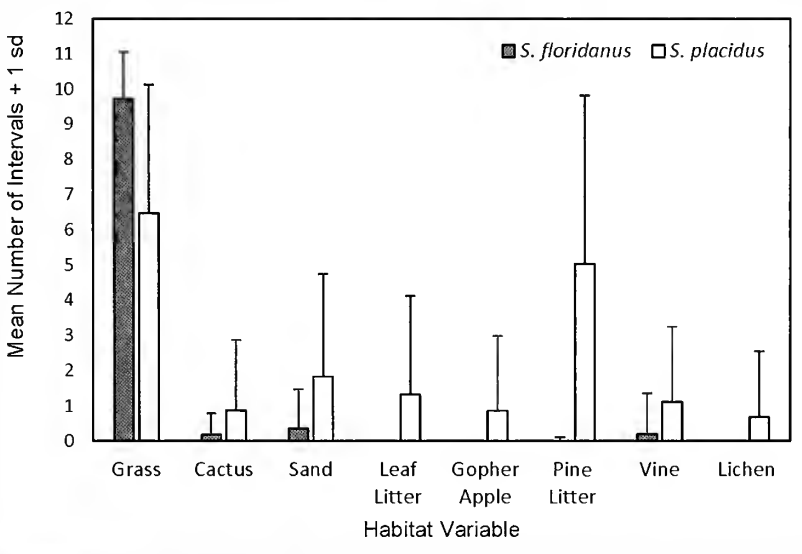

Figure 1.—Average number of 10 cm intervals per meter (+/- 1 s.d.) occupied by various habitat variables on transects through *S. floridanus* ($n = 112$) and *S. placidus* webs ($n = 149$).

Table 2.—Standardized discriminant function coefficients predicting the placement of *S. placidus* websites (n = 39) compared to random sites (n = 39), ranked using F scores. Due to lack of normality, the significance level is based on Mann-Whitney U tests. Pooled within-groups correlations between discriminating variable and standardized canonical discriminant functions show that only sand, lichen and gopher apple (negative values) predict random sites whereas all other variables (positive values) predict *S. placidus* websites.

Habitat variable	F	P	Canonical correlation coefficient
Cactus	22.22	<0.0001	0.734
Sand	5.11	0.0758	-0.356
Pine litter	2.15	0.1601	0.231
Vine	1.19	0.2190	0.172
Lichen	0.90	0.1447	-0.150
Leaf litter	0.52	0.5405	0.114
Grass	0.46	0.4697	0.107
Gopher apple	0.39	0.5995	-0.099

transects through random sites (n = 39), the only significant difference was in the presence of the cactus *O. humifusa* at *S. placidus* websites (Table 2; Fig. 2). Discriminant analysis found that *O. humifusa* was the primary predictor of *S. placidus* websites whereas open sand was more prevalent at random sites (Table 2).

DISCUSSION

This is the first in-depth examination of habitat associations of *Sosippus*. Anecdotal observations have recorded *S. floridanus* as occurring in pine flatwoods (Muma 1973) and high pine/palmetto (Brady 2007) plant communities, both of which are common to the entire Florida peninsula. The habitats at ABS are a mosaic of different plant communities including scrubby flatwoods and sand pine scrub (Abrahamson et al. 1984). The areas where we found *S. floridanus* are defined as scrubby flatwoods. Abrahamson et al. (1984) divides scrubby flatwoods into three phases: inopina oak (*Quercus inopina*), sand live oak (*Q. geminata*) and human-introduced Bahia grass. As reflected by the transect data, *S. floridanus* were largely associated with grass (Table 1, Fig. 1). Webs were found primarily in disturbed areas around the station grounds and along the margins of fire roads and fences, areas in which we never found *S. placidus*.

In contrast, *S. placidus* webs were located primarily in habitat defined as sand pine scrub/oak which is dominated by scrub oaks (*Quercus* spp.) and sand pine (*Pinus clausa*) (Abrahamson et al. 1984). Much of this habitat is located in some of the higher elevation areas of ABS, with very well drained soil and typically xeric adapted plants, such as the cactus *O. humifusa*. In the transects comparing *S. placidus* and *S. floridanus* websites, 24% of *S. placidus* webs were associated with *O. humifusa*; only 8% of *S. floridanus* websites were. In the comparison of *S. placidus* websites with random transects, 54% of *S. placidus* webs were associated with *O. humifusa*, while only 2% of random transects encountered it. The adaptive nature of this association becomes very clear when one tries to collect *S. placidus*. When associated with cactus, the funnel entrance is usually located at the base of the plant, making it very difficult for collectors, or more importantly, predators, to gain access to the spider without injury. Other scrub specific habitat variables (Myers 1990) associated with the presence of *S. placidus* but not with *S. floridanus* include gopher apple (*L. michauxii*), lichens (*Cladonia* spp) and lack of litter/open sand (Table 2).

This habitat separation of syntopic congeners is very similar to what has been found in studies of Florida species of the genus *Geolycosa* Montgomery, 1904 (Araneae: Lycosidae). Like *S. placidus*, *Geolycosa xera archboldi* McCrone, 1963 is found only in Highlands County (Marshall et al. 2000). *Geolycosa hubbelli* Wallace, 1942 is syntopic with *G. x. archboldi* in Highlands County, but similar to *S. floridanus*, has a wider distribution (Marshall et al. 2000). Comparisons of burrow site selection of these two species found that *G. x. archboldi* at ABS is strongly associated with xeric scrub habitats, similar to *S. placidus* (Marshall 1997; Marshall et al. 2000; Carrel 2003; Marshall & Martin 2011). Similar to *S.*

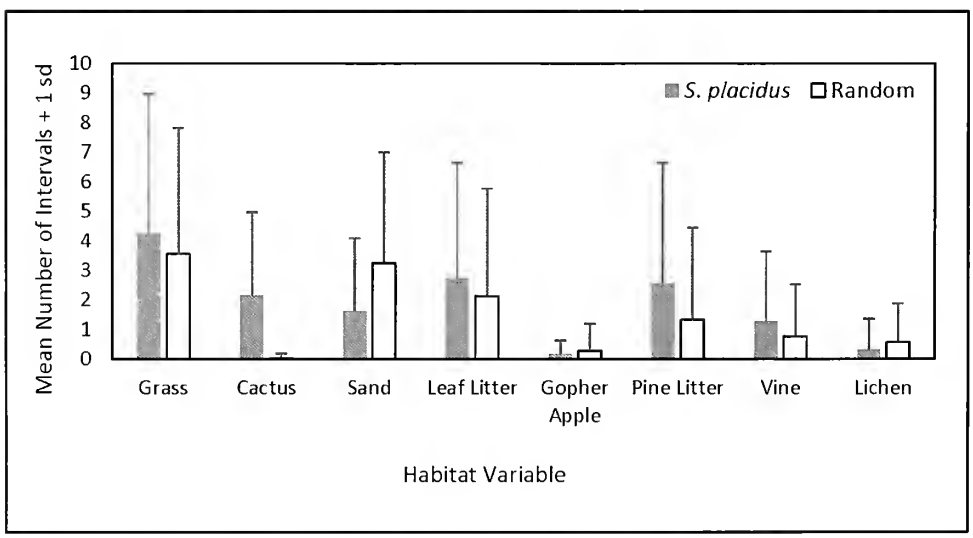

Figure 2.—Average number of 10 cm intervals per meter (+/- 1 s.d.) occupied by various habitat variables on transects through *S. placidus* websites (n = 39) and randomly selected sites (n = 39).

floridanus, *G. hubbelli* has a more generalized set of burrow site characteristics (Marshall et al. 2000; Marshall & Martin 2011).

Another similarity between the scrub endemics *S. placidus* and *G. x. archboldi* is limited dispersal ability. When *G. x. archboldi* spiderlings leave the maternal burrow, they disperse a very short distance before digging their own burrows (on average 55 cm), and if they relocate burrow sites again, move a short distance (on average 43 cm; Marshall 1997). *Sosippus placidus* is unique among wolf spiders in that the female exhibits extensive maternal care that qualifies as subsocial behavior. Brach (1976) conducted field and laboratory studies of this behavior at ABS (it should be noted, that when Brach published this work he was unaware that the spiders had been renamed by Brady (1972) and so he describes them as *S. floridanus*). He found that the young stayed in the maternal web for up to 5 months, subsequently dispersing into the immediate vicinity or building their own webs at the edge of the maternal web. The female captured and paralyzed prey for the young and fed very little during this period. (Brach 1976; Hodge, personal observation). Observations of *S. janus* Brady, 1972, which also has a very restricted ridge-associated distribution, suggests they may also exhibit subsocial behavior (Brady 2007; Hodge, personal observation). To date, no observations on maternal care and dispersal of *S. floridanus* exist. Many scrub endemics exhibit limited dispersal, presumably because they are adapted to the xeric conditions of scrub "islands" (Deyrup 1990). Our observations support the speculation of Brady (2007) that geographic isolation on Pleistocene islands resulted in the ecological specialization and limited dispersal behavior of *S. placidus*.

We initially hypothesized that the biogeographic distribution of *S. placidus* and *S. floridanus* reflected isolation of *S. placidus* to xeric dune systems during interglacial periods, with dispersal of *S. floridanus* down the peninsula during subsequent wetter and cooler periods. Based on morphological features Brady suggests that *S. floridanus* may be the most recently derived species (Brady 2007). Molecular work is currently underway to shed further light on the phylogenetic history of the Floridanus group of *Sosippus* (Hodge & Cushing, in progress). Still true, even a decade after Alan Brady wrote these words "The study of *Sosippus*, involving its phylogenetic relationships to other Lycosidae, its ecological and geographical distribution pattern, and its subsocial behavior, continues to raise many interesting questions" (Brady 2007, p. 55).

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New, sensitive behavioral assay shows scorpions are attracted to multiple wavelengths of light

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Abstract. Scorpions fluoresce a bright green when under ultraviolet light, but the functional significance of the fluorescence is still unknown. A major challenge in studying scorpion fluorescence is the lack of efficient methods for testing the behavioral photosensitivity of scorpions. We have modified previous assays to produce a more sensitive testing device. The apparatus consists of a circular track made of a small Petri dish nested inside a larger one, with an LED shining from the inner chamber across a small sector of the track. We monitored the scorpions' movements in the arenas under three light wavelengths: ultraviolet (399 nm), yellow-green (566 nm), and red (630 nm); all wavelengths were matched to a nighttime light intensity (0.01 irradians). We also tracked each animal's movements in the absence of light as a control. The animals were attracted to 399 and 566 nm light and also showed some attraction to 630 nm. Furthermore, earlier studies suggest that scorpion photoreceptors form a homogeneous population that has been physiologically shown to be maximally sensitive to green wavelengths. We hypothesize that the photoreceptor population might be somewhat responsive to red light too, suggesting that the photoreceptors may respond to a broad spectrum of light or that the photoreceptor population may not be as homogeneous as previously thought. The strong response to UV light, as has been seen in other behavioral assays, remains enigmatic. Overall, this new assay is more sensitive than previous assays for detecting scorpion photoresponse and will be useful for future studies.

Keywords: Photoreception, orientation, behavior, fluorescence

There is limited information on scorpion vision and light response. Scorpions typically have eight eyes: paired medial eyes that have lenses and may form images, and three pairs of lateral eyes that are sensitive to very low light levels (Schliwa & Fleissner 1980; Hjelle 1990). Physiological studies have shown a primary neural response of the medial eyes to green and a secondary response to UV light, but not red or infrared light (Fleissner & Fleissner 2001). Photosensitivity to green light has also been found in parts of the scorpion tail (Zwicky 1968, 1970a,b; Rao & Rao 1973).

Some behavioral studies have explored scorpions' responses to different wavelengths. Blass & Gaffin (2008) found that scorpions moved more sporadically under UV and green light compared to other wavelengths. Gaffin et al. (2012) found that the scorpions had a strong locomotor response to both 505 nm (green) and 395 nm (UV) with their eyes uncovered. When the eyes were covered with foil, the scorpions had a larger response to UV than to the green, and animals with uncovered eyes moved more under the green light than those with covered eyes under the same condition. Furthermore, Kloock et al. (2010) found that scorpions whose fluorescence was reduced by photobleaching moved between UV and dark portions of a test arena more frequently than control scorpions.

Our understanding of scorpion vision and the interplay between light, fluorescence, and behavior is hindered by a lack of sensitive behavioral assays. Previous approaches have monitored scorpion responses to light projected from above (Blass & Gaffin 2008; Kloock et al. 2010; Gaffin et al. 2012; Gaffin & Barker 2014). Here we report a new behavioral assay that tests scorpion responses to low-intensity, pure wavelengths of light directed across a discrete portion of a small circular track. We tested the response of scorpions to UV, yellow-green, red, and no light. We found that scorpions were most attracted to the yellow-green and UV light and somewhat attracted to red light; they did not show any behavioral preference in the no-light control.

METHODS

Animal collection and care.—The animals used in this study were 24 female *Paruroctonus utahensis* (Williams, 1968) that were collected near Monahans, Texas. These animals were individually kept in 3.8 L glass jars containing sand (to a depth of approximately 2.5–5.0 cm) and a small piece of clay pot. Diet consisted of one cricket every two weeks, and the sand was moistened three times a week with roughly 5 mL of water to keep the animals hydrated. A small heater maintained the temperature of the room containing the jars at 23–26°C, and the room was at 50–65% RH. The light-dark phase was 23:15–11:15 (light) and 11:15–23:15 (dark).

Arenas.—A plastic petri dish with a diameter of 55 mm was glued with chloroform to the center of a larger plastic petri dish with a diameter of 100 mm, making a circular track for the scorpions to walk. An LED was placed inside an L-shaped bracket, which was glued to the inside of the lid of the large dish so that when the lid covered the petri dishes, the LED touched the inner petri dish wall and directed its light across the track. We ran the LED's wire through a small hole in the lid and connected it to an op-amp circuit. We used a razor blade to scrape off three small ridges on the inside of the lid and used fine grit sandpaper to sand down the smaller inner petri dish to make a tight fit between the lid and dish. To ensure complete darkness in the arena, the entire lid of the large petri dish was covered with 2 layers of electrical tape. The bottom of the large petri dish also had a few layers of electrical tape on the outside of the side walls to keep out incoming light and to ensure a snug fit with the lid. We used a spectrometer (Ocean Optics USB4000 UV-VIS-E) to confirm that no light could enter the petri dishes.

We used four arenas for each set of trials, with each arena containing an LED (5 mm) emitting a different light wavelength: UV (399 nm, Newark), green (566 nm, LED-Tronics), red (630 nm Super Bright LEDs), and no light. We

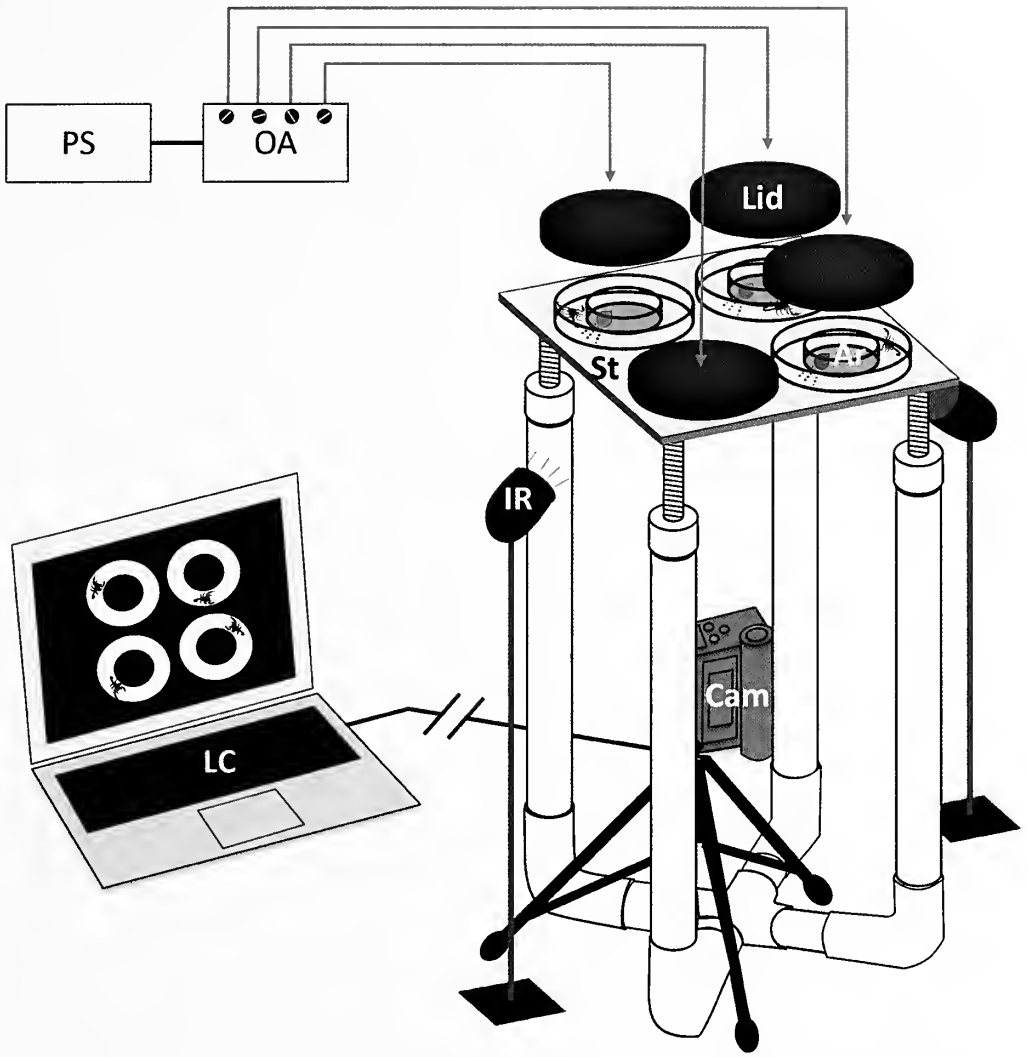

Figure 1.—Experimental apparatus. Four Petri dish arenas (Ar) sit atop a Plexiglas stage (St) supported by a frame made of PVC pipe with threaded extensions used for leveling. An infrared sensitive camera (Cam) monitors the trial from below and relays the feed to a laptop computer (LC) for storage and analysis. Supplemental infrared lamps (IR) direct light across the bottom of the arenas from the side to provide the light necessary to identify the scorpions on the camera (the camera's IR source was covered to reduce glare). LEDs are situated inside the inner Petri dish and direct light radially across the track that holds the scorpion. The LEDs, which are glued to the lids of the dishes, are pictured in their positions after the lid is shut. Wires lead from a power supply (PS) and operational amplifier circuit (OA) to control LED intensity.

used the spectrometer to measure (through the plastic petri dish wall) the LED wavelengths to the nearest nm. The no-light control was identical to the other arenas in that it still contained a bracket and an LED, but the polarity of the circuit was reversed to mimic the potential heat that may be radiating from the LED, further normalizing the experiences between the scorpions. We adjusted a variable resistor on the circuit board to set each light at 0.01 ± 0.005 $\mu W/cm^2/nm$ (as verified by the spectrometer). We chose this irradiance based on scorpion responses in a previous study (Gaffin & Barker 2014).

Apparatus.—We placed the arenas on top of a Plexiglas table supported with PVC legs (Fig. 1) and filmed the arenas from below with an IR sensitive camera (Sony Handycam CCD-TRV16). To reduce the glare on the table, we covered the IR source on the camera with electrical tape and used two additional IR spotlights (Defender) from the side. The spotlights were covered with Parafilm to further scatter the light. The irradiances of the spotlights measured from the distance of the arenas were 0.449 $\mu W/cm^2/nm$ and 0.502 $\mu W/cm^2/nm$, respectively, with a peak wavelength of 845.75 nm. The camera was connected to a computer with a video capture program (Sony Elgato). The computer was about 60 cm away from the apparatus and the screen was oriented away from the apparatus.

The location of each dish and LED color was marked on the table to standardize the placement of the arenas between scorpions. The LED inside each dish pointed towards a unique cardinal direction. The initial pattern was randomly assigned; from there, we rotated the arenas counterclockwise 90° with each trial set.

Trial protocol.—With only a single red light on in the room (oriented away from the apparatus; peak 659.5 nm; 0.0762 $\mu W/cm^2/nm$ at arenas), the four arenas were cleaned with Kimwipes and 70% ethanol. While the arenas were left to dry, the computer and the camera were turned on. Each scorpion was taken out of its jar and placed on the side of the track opposite the LED. The lid was placed on the arena, making sure that the Petri dishes

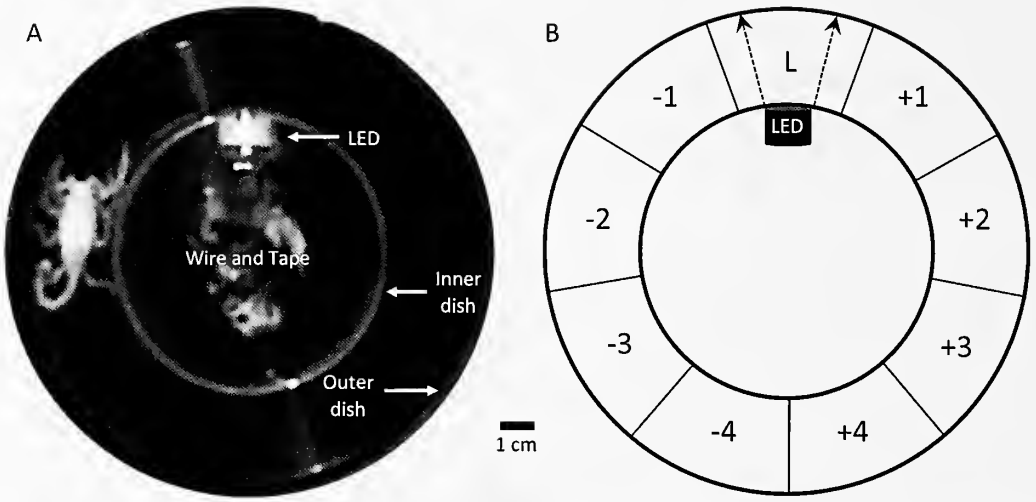

Figure 2.—Close-up and division of arena. A. Scorpions were initially placed in the side of the arena opposite the LED. B. The X and Y coordinates of scorpion location (as determined by MATLAB) were converted into degrees, and each location corresponded to one of the nine sectors of the track; the sector labels correspond to the plots shown in Figure 3. The lines at about 5 and 11 o'clock in the image to the left are glare from the IR beams directed across the bottom of the dishes.

were flush with the lid. The arena was placed in its corresponding spot on the table, facing the appropriate cardinal direction, and the red room light was turned off. The video capture program was set to record for 60 minutes. Once the IR spotlights and LEDs were turned on and the computer screen was turned off, the recording was immediately started. After the 60-minute recording, the red room light was turned on, the video was saved to the computer, and the spotlights and LEDs were turned off. The scorpions were returned to their jars and the arenas were cleaned once again with 70% ethanol. Then, the next set of scorpions was placed in the arenas and the procedure repeated.

The experiment was conducted for three days (Monday, Tuesday, and Thursday) in each of four weeks, with two consecutive trial sets recorded each day (total of 24 scorpions). Each trial set consisted of four scorpions, one for each light variable. Every scorpion experienced each light variable only once, and each animal had a week-long break between trials. After two sets of trials (or one experimental day), the arenas were rotated clockwise approximately 90 degrees to minimize any room effects, such as the Earth's geomagnetic field. The order the scorpions experienced the lights was also randomized.

Two minor departures from the specified schedule and temperature conditions occurred. The first two sets of animals repeated their first light variable twice due to a software glitch that prevented saving the video file of the animals. These animals were still given a week off to reduce acclimation to the test environment. Another deviation from the stated conditions happened unexpectedly in the middle of the second week of trials when the temperature increased from 26°C to 28°C. The temperature returned to 26°C at the beginning of the fourth and final week of trials.

Location mapping / analysis.—Only the scorpions that entered the light sector at least once for each of the four light variables were considered legitimate and analyzed for this assay. Once those scorpions were identified, the footage from each hour-long recording was time-lapsed to 1 frame / 2 seconds (using *iMovie*, Apple Inc.) and condensed further with a MATLAB script to one frame every 3.40 seconds. Another MATLAB script was used to determine the X and Y coordinates of the location of each scorpion based on the centroid of blob generated by the frame-by-frame subtraction. The size of the centroid was not artificially enlarged near the LED light sources for two reasons: (1) each LED produced a narrow range of wavelengths, which the IR-sensitive camera did not pick up; and (2) the arenas were illuminated and recorded from below, so the IR illumination was distributed evenly across the entire lower surface of each arena. Only frames that differed from the previous frame were retained for analysis (i.e. non-movement frames were removed). Several MATLAB functions were used to convert the linear coordinates for legitimate trials into degrees of a circle. We then computed the frequencies of the scorpions' occupancy in nine equal sectors as shown in Fig. 2 (with the light orientation determining the midpoint of the light-indexed sector). For our final analysis, we averaged the + and − sectors (essentially ignoring differences between left and right of the light and focusing on proximity to the light). Using the average corrected for the fact that there is only one L sector, but two sectors each for +/- 1, +/- 2 etc. This reduced the number of categories being tested in the sector comparison to five.

Thirteen of the 24 scorpions entered the light sector at least once for all four light conditions. We used a repeated measures ANOVA and a Tukey-Kramer multiple comparisons test to assess the average time that these scorpions spent in the light sector compared to the other four sectors and the average time spent in the light sector among conditions (with significance set at $P<0.05$). We used Prism 6 statistical software (Graph Pad Software, Inc., San Diego, CA, U.S.A.) for our statistical analyses.

RESULTS

Most animals moved readily in the behavioral arenas. Though only 13 of the 24 animals ventured into the light-containing sector for all four light conditions, animals moved away from their starting position in 84 of the 96 trials (87.5%). The animals' typical walking pattern consisted of a few

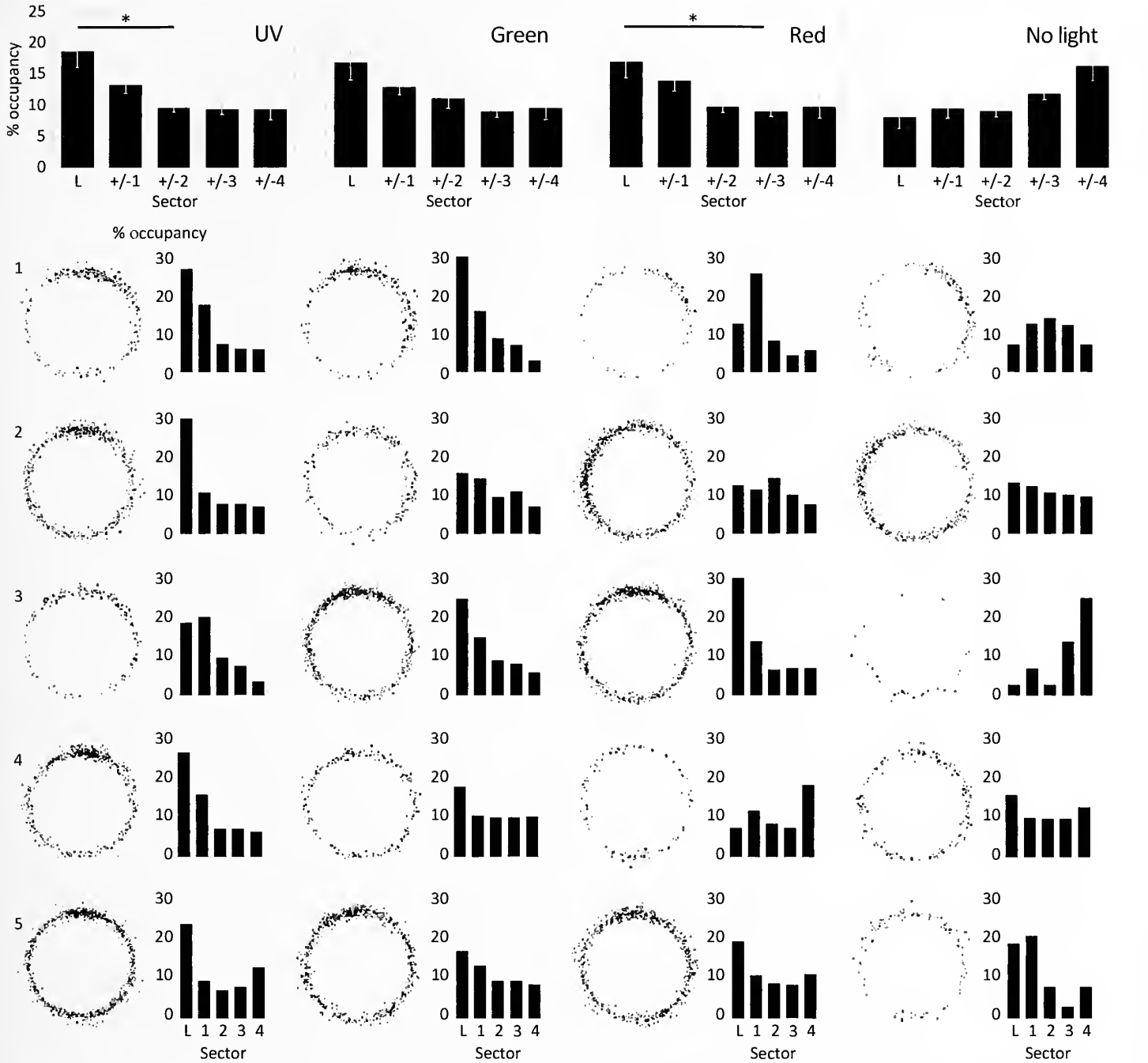

Figure 3.—Analysis of scorpion positions relative to light. Rows 1-5 show plots of activity in Petri dish arenas by 5 of the 13 animals that completed at least one full lap of the arena under all four light conditions during the 1 h test periods, arranged by animal and light condition. Dots represent animal location every 3.40 s. All arenas have been oriented so that the light direction is toward the top of the figure. Histograms next to plots show occupancy by arena sector; L is the light sector and +/- 1 being the average occupancy of the sectors immediately adjacent to the light sector, +/- 2 the average occupancy of sectors two away from the light sector, etc. The histograms at the top of the figure show mean occupancy (+SE) of these five sectors by all 13 animals under each light condition. Significant sector differences ($P<0.05$) compared to the light sector based on Tukey-Kramer multiple comparisons test are indicated by "*".

forward steps followed by a pause lasting a few seconds. While walking, their anterior prosoma was typically angled toward the periphery of the outer Petri dish, with their pedipalps making intermittent contact with the outer wall. Also, we noticed numerous U-turns and rapid walking motions that indicated that animals were free to move in any desired direction if they chose to do so.

Plots of the data for 5 of the 13 animals that completed at least one full lap of the arena under all four light conditions are shown in Fig. 3. We have rotated the plots so that the LED positions are to the top of the figure (even though the LEDs faced various cardinal directions during the trials). The histogram next to each plot shows percent occupancy of the five designated sectors. Over all 13 animals that completed at

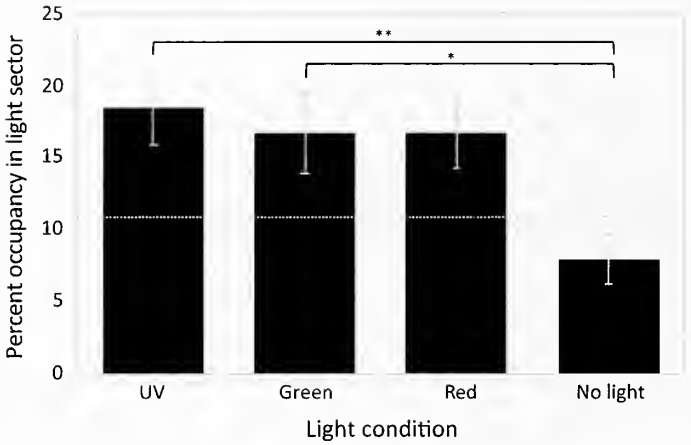

Figure 4.—Summary of behavioral responses to light. Mean (+SE) percent occupancy in light sector by condition; the dotted line indicates level of random choice (100/9 = 11.1%). Asterisks indicate conditions that are statistically different (** = $P<0.01$, * = $P<0.05$).

least one full lap of the arena under all four light conditions, the sector with the greatest occupancy coincided with the light sector in eight of the UV trials, nine of the green trials, seven of the red trials, and two of the no-light trials. Mean percent quadrant occupancy by light condition for all 13 animals is shown at the top of Fig. 3. Significant differences in the distribution of sector occupancy were seen for the UV and red light conditions (ANOVA, repeated measures, df = 12, UV: $F = 5.855$, $P = 0.0125$; green: $F = 2.659$, $P = 0.0899$; red: $F = 3.663$, $P = 0.0317$; no light: $F = 3.649$, $P = 0.0630$). Occupancy of the light sector was significantly higher than sector 2 for the UV trials and sector 3 for the red trials (Tukey-Kramer multiple comparisons test). The plot of sector occupancy for the control trials appears skewed toward the sectors farthest away from the LED-containing sector. This is likely because the animals were always started opposite the LED and were not drawn repeatedly to the LED-containing sector as were the light-stimulated animals. When the sector occupancy of the 13 control trials was calculated based on a consistent geocentric orientation, no significant differences were detected among the sectors, indicating that no extraneous cues affected orientation in the test arenas.

Fig. 4 shows the mean percent occupancy in the light sector for the three light conditions and the control (the expectation for random occupancy is indicated by the horizontal dotted line at 11.1% (100% divided by 9 sectors). The repeated measures ANOVA P value was 0.0167 ($F = 4.383$, df = 12) and considered significant. Significant behavioral differences also existed between UV and no light ($P<0.01$) and green and no light ($P<0.05$) conditions.

DISCUSSION

In this assay, we found significant behavioral responses to yellow-green and UV light and also a response to red. In all of the arenas but the control, the scorpions lingered in the light-containing sector and oriented toward the light, which is different from their typical light-avoiding behavior.

These results differ from what we expected, based on previous physiological and anatomical studies. Gaffin et al. (2012) found no response to the 565-nm wavelength in their study and they used a higher irradiance (0.15 $\mu W/cm^2/nm$). The physiological response of the median and lateral eyes of *Androctonus australis* (Ewing, 1928) peaks in the blue-green range (500 nm) and falls to half this peak response by 565 nm and essentially no response by 630 nm (Fleissner & Fleissner 2001). Furthermore, a secondary "shoulder" of responsiveness (about 60% of maximum) exists in the 350–400 nm range. Curiously, when illuminated with UV light in the 350–400 nm range, the peak fluorescence emission also falls in the 500-nm range (Fasel et al. 1997). In addition, Fleissner & Fleissner (2001) suggested that scorpions have a homogeneous population of photoreceptors, indicating that scorpions are color blind. However, the results of this assay suggest that scorpions may detect a broader spectrum of light than initially thought. It is possible that the photoreceptors are homogeneous, but respond to a broad spectrum of wavelengths, or that the photoreceptors are heterogeneous, and the scorpions have more than one response maximum.

Although behavioral patterns were not further quantified, we did notice that responsive animals often turned toward the LED, and their pedipalps and forward pairs of legs appeared to make deliberate contact with the inner wall (Fig. 5). Such animals often reversed their course, repeating this movement towards the LED several times; some animals even contorted their bodies in front of the LED, appearing to survey the floor and ceiling of the chamber. The motivations for this behavior suggest an opportunity for follow-up studies.

The configuration of previous behavioral studies used light that flooded the entire arena from above to detect locomotor changes to different wavelengths (Gaffin et al. 2012). In the current study the animals only experienced light when they passed through the light-containing sector of the arena and they could adjust their exposure by their behavior. Scorpions have a protective pigment that shifts on and off the eyes across a day and changes visual sensitivity up to 4 log units (Fleissner & Fleissner 2001). The animals of this and previous studies were dark adapted and the sunset irradiance of 0.01 $\mu W/cm^2/nm$ that we used here was also used in the previous configuration. It is possible that the constant light of previous studies light-blinded the animals and rendered them less sensitive; the limited exposure of the current configuration may have allowed for responses to the longer wavelengths.

There were a few challenges with some components of this study. One concerns the starting position of the scorpions. While each scorpion was initially placed on the side of the arena opposite the LED, some scorpions scurried along the track and immediately experienced the light, whereas the animals that remained motionless started in the dark. To normalize the experience among scorpions, the arenas could be tilted on their sides to shift the scorpions away from the LED until the recording is started. Another issue is the lack of movement from some scorpions, which could be mitigated somewhat by elevating the temperature of the room or the test chambers.

Given these results, future assays can use the 0.01 irradians to explore behavior and perception. It will be interesting to test many additional wavelengths, including below 399 nm and at regular intervals from 400 to 630 nm and beyond. In addition, sensory ablation studies and the use of photo-

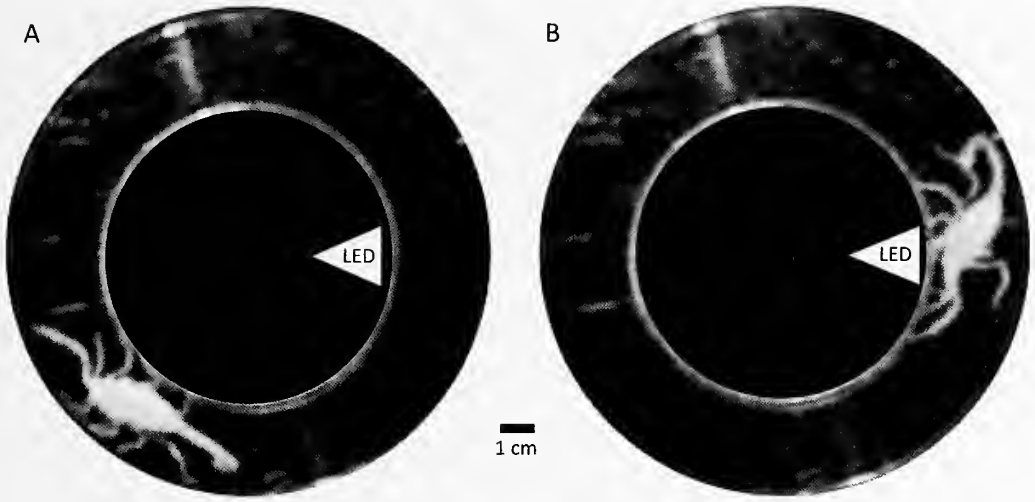

Figure 5.—Typical animal body position in front of and away from LED. A. Animal away from LED with pedipalps surveying outer wall. B. Animal in front of LED (UV in this case) with pedipalps, front legs, and anterior prosoma against inner wall.

bleached scorpions (Kloock 2009) can be used to investigate the contributions of ocular and extra-ocular (Zwicky 1970a,b) photodetection in mediating these responses. Most importantly, the connection to the scorpion's fluorescence, behavior in its natural habitat, and other aspects of its biology need to be better understood and should be investigated.

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Systematics of the giant spiny trapdoor spiders of the genus *Gaius* Rainbow (Mygalomorphae: Idiopidae: Aganippini): documenting an iconic lineage of the Western Australian inland arid zone

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Abstract. The aganippine spiny trapdoor spiders of the genus *Gaius* Rainbow, 1914 are revised. Seven new species are described from Western Australia: *G. aurora* sp. nov., *G. austini* sp. nov., *G. cooperi* sp. nov., *G. hueyi* sp. nov., *G. humphreysi* sp. nov., *G. mainae* sp. nov. and *G. tealei* sp. nov. The type species, *G. villosus* Rainbow, 1914, is re-illustrated and re-diagnosed, and molecular data for six (of eight) species and six genes are analyzed with Bayesian methods. Species of *Gaius* are iconic denizens of the Western Australian inland arid zone, renowned for their large size and extreme longevity. We here document the known diversity and conservation status of these spiders, and summarize their unusual biology and phenology.

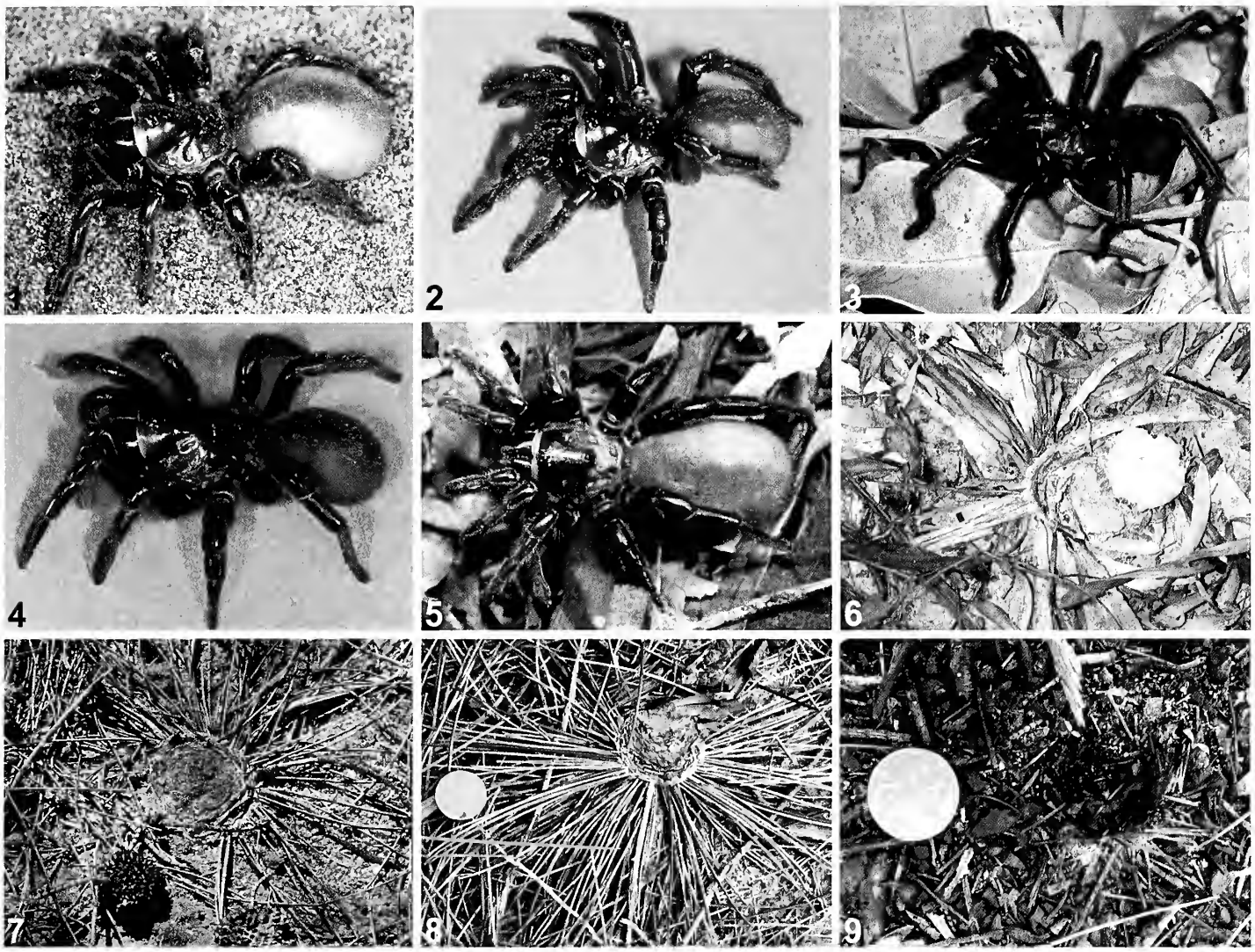
Keywords: Taxonomy, subfamily Arbanitinae, *Anidiops*

http://zoobank.org/?lsid=urn:lsid:zoobank.org:pub:96416C0A-7D1C-4C56-82FE-85A1E46528E1

The giant spiny trapdoor spiders of the Western Australian endemic genus *Gaius* Rainbow, 1914 (Figs. 1–10) are iconic denizens of the inland arid zone, renowned for their large size and extreme longevity. Indeed, excluding Theraphosidae, *G. villosus* Rainbow, 1914 is one of Australia's largest mygalomorph spiders; their ornate burrows (Figs. 6–9) can be found in remnant woodlands throughout much of inland Western Australia, and the very large males (Fig. 3) are often found wandering after summer thunderstorms. *Gaius villosus* is also among the best known of Australia's Idiopidae, thanks to a decades-long demographic study undertaken by Barbara Main in the central Wheatbelt bioregion (e.g., see Main 1978, 1987). Here, burrows were first tagged in 1974, and individual spiders followed thereafter over the course of their lifetimes. As a result of this work, unprecedented in its temporal perspective, we now know that *G. villosus* is one of the world's longest-lived spiders, with some females able to survive for over 40 years in the wild (Main 1978, 1987, unpubl. data). This ability of large individual 'matriarchs' to persist for long periods of time in highly ephemeral arid habitats, is a survival strategy utilized by a number of idiopid taxa, including species of *Idiosoma* Ausserer, 1871. Females breed only when and if conditions are suitable, and when adequate protein is available for the development of eggs. In doing so, they have managed to colonize some of the harshest landscapes in Western Australia, and *Gaius* is one of only four idiopid genera known to occur as far north as the Pilbara bioregion (the others being *Bungulla* Rix, Main, Raven & Harvey, 2017, *Euoplos* Rainbow, 1914 and *Idiosoma*) (see Rix et al. 2017d).

The genus *Gaius* has had a rather confusing taxonomic history, and for 60 years it was not even recognized as valid following its synonymy with *Anidiops* Pocock, 1897 by Main (1957), the latter itself now a synonym of *Idiosoma* (as per Rix et al. 2017d). This synonymy of the then monotypic *Gaius* with the similarly monotypic *Anidiops* was founded on the perceived significance of sclerotized abdominal sigilla, or in the case of *G. villosus* and *A. manstridgei* Pocock, 1897, their absence relative to *Aganippe* O. P.-Cambridge, 1877 (= *Idiosoma*) and *Idiosoma*. We now recognize that unsclerotized sigilla are symplesiomorphic for Arbanitinae, and therefore of no diagnostic or phylogenetic value in most taxa. Indeed, sclerotized sigilla likely evolved only once, in the common ancestor of *Idiosoma* and *Eucanippe* Rix, Main, Raven & Harvey, 2017 (Rix et al. 2017b), and were secondarily lost in the *manstridgei*-group (= *I. manstridgei* and its closest relatives). As a result, Rix et al. (2017d) revised the status of *Gaius* following detailed molecular phylogenetic analysis of the Australasian idiopid fauna (Rix et al. 2017b), and highlighted the affinities of the genus to *Eucyrtops* Pocock, 1897 and *Bungulla* (Fig. 10). By this time, it was also clear that *Gaius* was not just a monotypic branch of Aganippini, as a number of new species were known from collections, and preliminary molecular data similarly pointed to a multi-species lineage that was distributed north to at least the Pilbara (Castalanelli et al. 2014). Documenting this diversity is therefore the aim of our study, not least because the conservation of these large and iconic invertebrate predators (Rix et al. 2017b) is dependent on a robust taxonomy.

This paper is the fifth in a series of revisionary works to describe Western Australia's known species of *Gaius*, *Bungulla* (see Rix et al. 2017d, 2018b), *Cataxia* Rainbow, 1914 (see Rix et al. 2017a), *Eucanippe* (see Rix et al. 2018a), *Eucyrtops*, *Euoplos* and *Idiosoma* (see Rix et al. 2017d, in part). Seven new species of *Gaius* are here described, taking the total number of species in the genus to eight.



Figures 1–9.—Live habitus images and burrows of *Gaius* from Western Australia. 1–3, *G. villosus* Rainbow, 1914, live habitus images: 1, female (WAM T132736) from Minnivale Nature Reserve; 2, female (WAM T141124) from Vermin Proof Fence, NW. of Eurardy; 3, male from Wongan Hills. 4, Live habitus image of female *G. cooperi* sp. nov. (WAM T144017) from Westralia Conservation Park. 5, Live habitus image of female *G. austini* sp. nov. (WAM T116013) from Credo Station. 6–8, Burrows of *G. villosus*: 6, from Minnivale Nature Reserve; 7, from Bungulla Nature Reserve; 8, from Vermin Proof Fence, NW. of Eurardy, with Australian one dollar coin (diameter 25 mm) for scale. 9, Burrow of *G. cooperi* sp. nov. from Westralia Conservation Park, also with one dollar coin for scale; the twig-lines have been mostly burnt in a bushfire but remnants remain. Note the large, heavily-built and dark-colored somatic morphology of the spiders, and the radiating twig-lines characteristic of this genus. Images 1, 2, 4–6, 8, 9 by M. Harvey; 3 by M. Rix; 7 by D. Joliffe, used with permission.

METHODS

Morphological methods.—Morphological methods, including the format of species descriptions and imaging techniques, follow Rix et al. (2017d, 2018a, b), with measurements in millimeters and species presented in alphabetical order (following the type species). All species are distinguished and diagnosed according to a generalized species concept, whereby morphological and (where possible) molecular data are combined to provide the operational criteria for distinguishing "separately evolving metapopulation lineages" (de Queiroz 2007: 880). Most available male specimens of *Gaius* were illustrated for this study, either within the primary numbered plates or, for additional (non-holotype) specimens, as an 'Atlas' series of more rapidly assembled single-shot images in four standard views (see Supplementary File 1, online at http://dx.doi.org/10.1636/JoA-S-17-079.sn1). The latter are included for ease of comparison to the type specimens, to directly illustrate the subtle morphological variation in key characters typical of Mygalomorphae, and to provide a comprehensive digital compendium of the material available in collections. For records with multiple specimens per vial (some vials of which included multiple registration numbers), only a single exemplar specimen was imaged. In the case of *G. villosus*, for which > 100 records were available, a geographically and morphologically representative selection of 30 specimens was imaged (see Supplementary File 1, online at http://dx.doi.org/10.1636/JoA-S-17-079.s1). Females of *Gaius* species are difficult to identify using morphology alone, however sequence data and/or syntopic collection with known

males are usually adequate for identification purposes. Maps were generated using the online Atlas of Living Australia (online at http://www.ala.org.au/), and are reproduced under a Creative Commons Attribution 3.0 Australia license.

Specimens are lodged at the Western Australian Museum, Perth (WAM), the Australian Museum, Sydney (AMS) and the Queensland Museum, Brisbane (QMB), and the following abbreviations are used throughout the text: ALE, anterior lateral eye/s; AME, anterior median eye/s; *COI*, cytochrome *c* oxidase subunit I; *CYB*, cytochrome b; IBRA, Interim Biogeographic Regionalisation of Australia Version 7 (online at https://www.environment.gov.au/land/nrs/science/ibra); ITS1–2, internal transcribed spacer 1–2; *MRPL45*, 39S ribosomal protein L45 mitochondrial; PLE, posterior lateral eye/s; PME, posterior median eye/s; *RPF2*, ribosome production factor 2 homolog; RTA, retrolateral tibial apophysis (of male pedipalp); *XPNPEP3*, probable Xaa-Pro aminopeptidase 3. For readability and ease of diagnosis, 'sp. nov.' epithets are removed from the main text after the key to species.

Molecular methods.—Nucleotide sequences for six genes (*CYB*, ITS1–2, *MRPL45*, *RPF2*, *XPNPEP3*) were generated for 21 specimens of *Gaius* for which tissue was available, using a next-generation parallel tagged amplicon sequencing (TAS) approach, described in detail by Rix et al. (2017b). For six additional specimens (codes: NCB_023–028), sequences were generated using standard bi-directional sequencing of polymerase chain reaction (PCR) amplicons, as per Castalanelli et al. (2017), using the same primers as Rix et al. (2017b). The gene *COI* was excluded from the main analyses (Fig. 13), due to the presence of a possible pseudogene and often multiple banding in PCRs. However, a separate supplementary 'COI' analysis of verifiable legacy *COI* data was performed, to: (i) confirm the identity of the female and juvenile specimens barcoded by Castalanelli et al. (2014); and (ii) to link the male holotype of *G. humphreysi* sp. nov. (WAM T96563), for which a *COI* sequence was successfully amplified, but for which nuclear data were otherwise lacking. This 'COI' analysis included the 46 specimens of *Gaius* sequenced by Castalanelli et al. (2014), the male holotype of *G. humphreysi* sp. nov., and eight other specimens of *Gaius* for which verifiable barcodes were also available (at least one for each of the six sequenced species) (see Supplementary File 5, online at http://dx.doi.org/10.1636/JoA-S-17-079.s5).

Outgroup sequences were obtained from data previously published by Rix et al. (2017b, 2018b). The ultimate outgroup for the molecular analyses was the diplurid spider *Cethegus fugax* (Simon, 1908), and an undescribed species of *Prothemenops* Schwendinger, 1991 was also included in all analyses. For the 'FULL' dataset (all six genes) and 'NUCLEAR' dataset (five nuclear genes), the three specimens of *Bungulla biota* Rix, Main & Harvey, 2018 sequenced by Rix et al. (2018b) were added, as was one specimen of *B. gibba* Rix, Main & Harvey, 2018, the latter also sequenced by Rix et al. (2018b). In total, 27 specimens were analyzed for the 'FULL' dataset (see Supplementary File 2, online at http://dx.doi.org/10.1636/JoA-S-17-079.s2; excluding taxa without mitochondrial *CYB* sequences), 33 specimens were analyzed for the 'NUCLEAR' dataset (see Supplementary File 3, online at http://dx.doi.org/10.1636/JoA-S-17-079.s3), and 56 specimens were analyzed for the supplementary 'COI' dataset (see Supplementary Files 4–5, online at http://dx.doi.org/10.1636/JoA-S-17-079.s4 and http://dx.doi.org/10.1636/JoA-S-17-079.s5). For each specimen sequenced, DNA voucher codes and GenBank accession numbers are provided next to repository registration numbers in the material examined section for each species (below), in the form: [Registration^{DNA_Voucher_Code}; GenB–GENE–No., etc.].

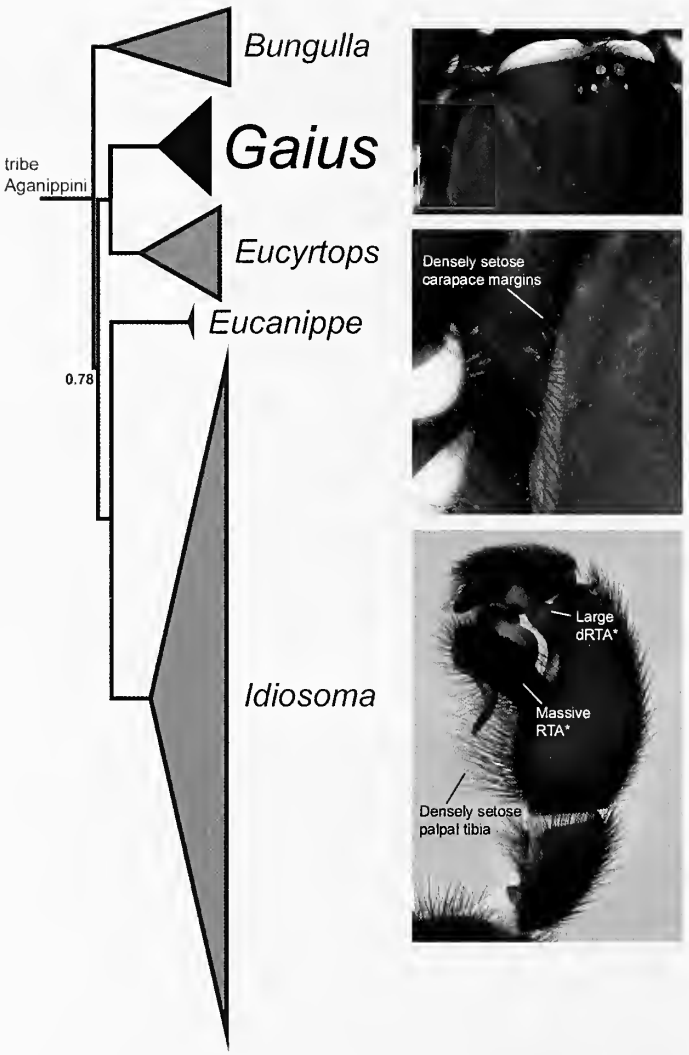


Figure 10.—Summary phylogeny of the tribe Aganippini, from the 'FULL' 12-gene Bayesian analysis of Rix et al. (2017b), showing the phylogenetic position of *Gaius*. Inset images show the male lateral carapace margin (boxed region enlarged in panel 2) and retrolateral pedipalp of *G. villosus* Rainbow, 1914, and the four apomorphies characteristic of males of the genus. Highlighted (*) characters are present in most, but not all, species.

Individual gene alignments were conducted in Geneious R6 (Biomatters Ltd.; online at http://www.geneious.com/) using the MAFFT v7.017 plugin with default parameters (Katoh & Standley 2013), and these alignments for the six genes were concatenated to generate the 'FULL' dataset (Supplementary File 2, online at http://dx.doi.org/10.1636/JoA-S-17-079.s2) which included all available data, and the 'NUCLEAR' dataset (Supplementary File 3, online at http://dx.doi.org/10.1636/JoA-S-17-079.s3) which included only the five nuclear

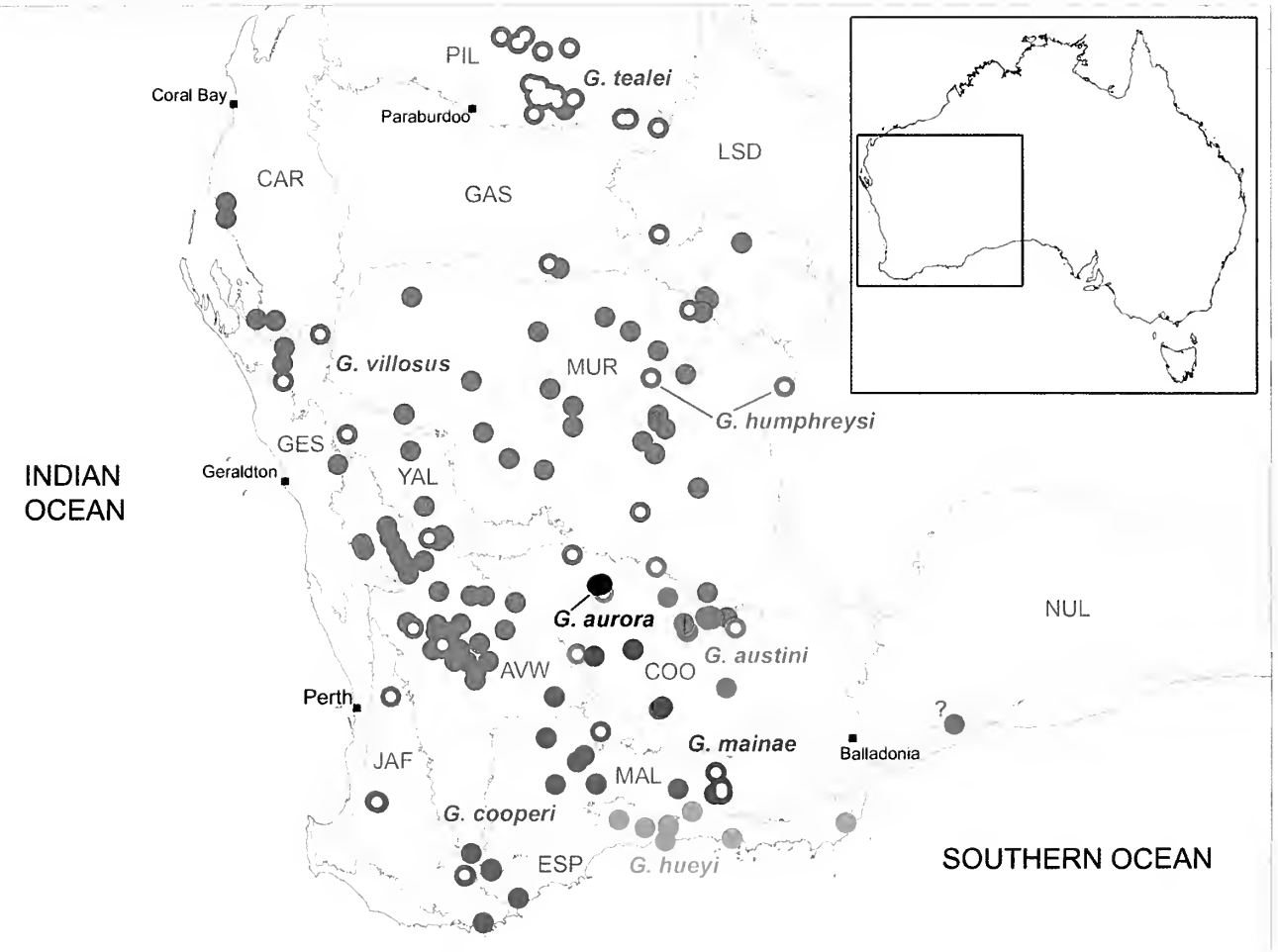


Figure 11.—Map showing collection records of *Gaius* from Australia, color-coded according to species. Open circles denote specimens sequenced for the molecular analyses, and note the specimen from east of Balladonia ("?"), only tentatively assigned to *G. humphreysi* sp. nov. Relevant IBRA 7.0 bioregional acronyms are as follows: AVW, Avon Wheatbelt; CAR, Carnarvon; COO, Coolgardie; ESP, Esperance Plains; GAS, Gascoyne; GES, Geraldton Sandplains; HAM, Hampton; JAF, Jarrah Forest; LSD, Little Sandy Desert; MAL, Mallee; MUR, Murchison; NUL, Nullarbor; PIL, Pilbara; YAL, Yalgoo.

genes. PartitionFinder Version 1.1.1 (Lanfear et al. 2012) was used to choose an optimal partitioning scheme, favoring an eight partition model for the 'FULL' dataset; for the 'NUCLEAR' dataset, two mitochondrial partitions were excluded. For the 'COI' dataset, a parameter-rich (GTR + G) model was applied to each codon position. All three datasets (Supplementary Files 2–4, online at http://dx.doi.org/10.1636/JoA-S-17-079.s2, dx.doi.org/10.1636/JoA-S-17-079.s3, and dx.doi.org/10.1636/JoA-S-17-079.s4) were analyzed in MrBayes Version 3.2.6 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003) via the CIPRES Science Gateway (Miller et al. 2010), with substitution model parameters estimated independently for each partition ([Unlink tratio = (all) pinvar=(all) shape=(all) statefreq=(all) revmat=(all)]) and rates allowed to vary across partitions ([Prset applyto=(all) ratepr=variable]). Four Markov Chain Monte Carlo (MCMC) chains were run for 10 million generations for each analysis, sampling every 1000 generations, with the first 10% of sampled trees discarded as 'burnin' ([burnin = 1000]). Summary statistics of estimated parameters, including ESS values, were assessed using Tracer Version 1.6 (Rambaut et al. 2014), and FigTree Version 1.4.2 (online at http://tree.bio.ed.ac.uk/software/figtree/) was used to visualize 50% majority-rule consensus trees (Fig. 13, Supplementary File 5, http://dx.doi.org/10.1636/JoA-S-17-079.s5).

Phenology.—Data on the phenology of each species were assembled for those specimens for which a specific month of collection could be ascertained. In total, 189 specimens of *Gaius* were included in the statistics (Fig. 12). Data for each species and for the genus as a whole were analyzed separately by month of collection, with reported *N* values denoting the number of males included in the phenological assessment (i.e., excluding males collected across a range of reported months).

Conservation assessments.—The conservation status of each species of *Gaius* was assessed using a standard International Union for the Conservation of Nature (IUCN) approach, similar to that applied by Harvey et al. (2015) for migid trapdoor spiders of the genus *Bertmainius* Harvey, Main, Rix & Cooper, 2015, and by Rix et al. (2017a) for the idiopid genus *Cataxia* in south-western Australia. As long-term data on population reductions (Criterion A), population sizes or

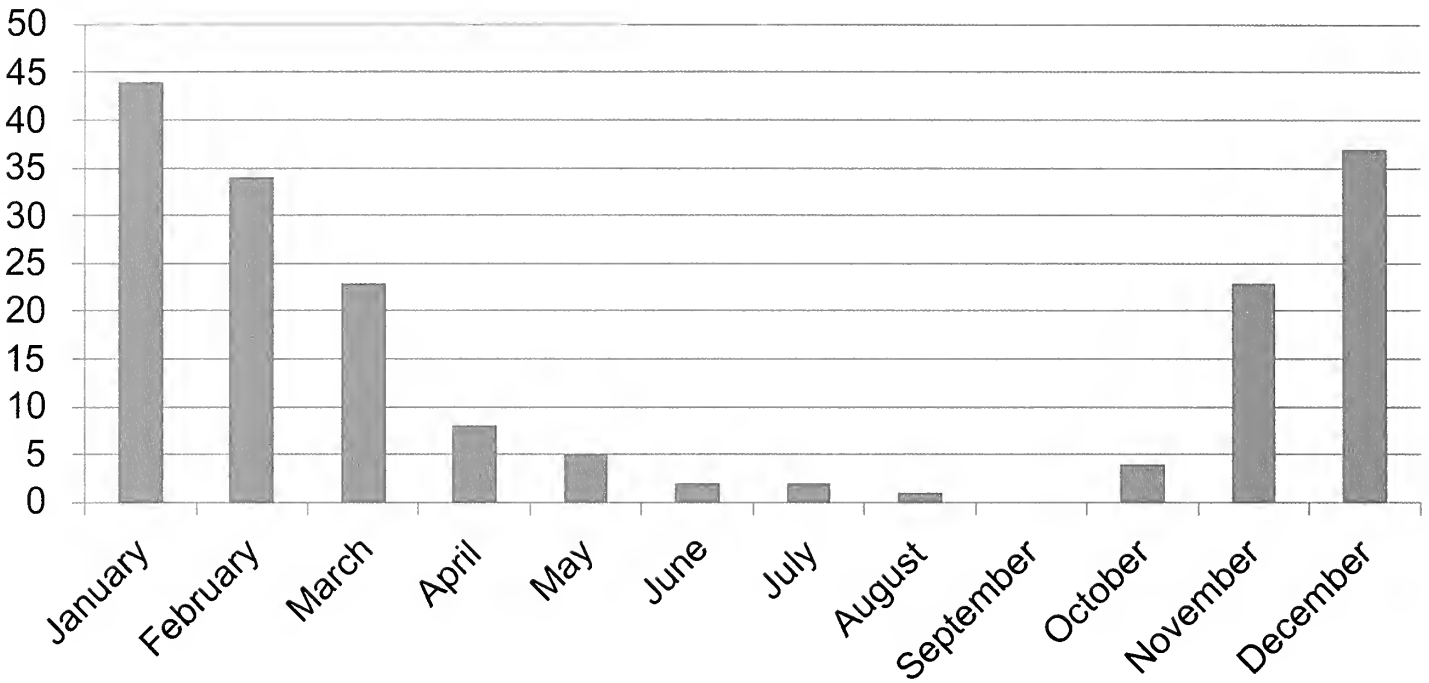


Figure 12.—Graph summarizing phenology data for 183 male specimens of *Gaius* for which an accurate collection month could be ascertained. The y-axis denotes the number of specimens collected per month. Note the peak of activity in the warmest months of November–March.

declines (Criterion C) or the number of mature individuals in any one population (Criterion D) were not available, we assessed all taxa using information on their geographic range (Criterion B) (as per Rix et al. 2017a), the latter calculated using area polygons in Google Earth Pro (Google Inc.). These assessments therefore focused on the extent of occurrence of each species, the area of occupancy within that range, and the health or otherwise of occupied habitats. Individual assessments are listed for each species under their relevant entry in the Systematics section (below).

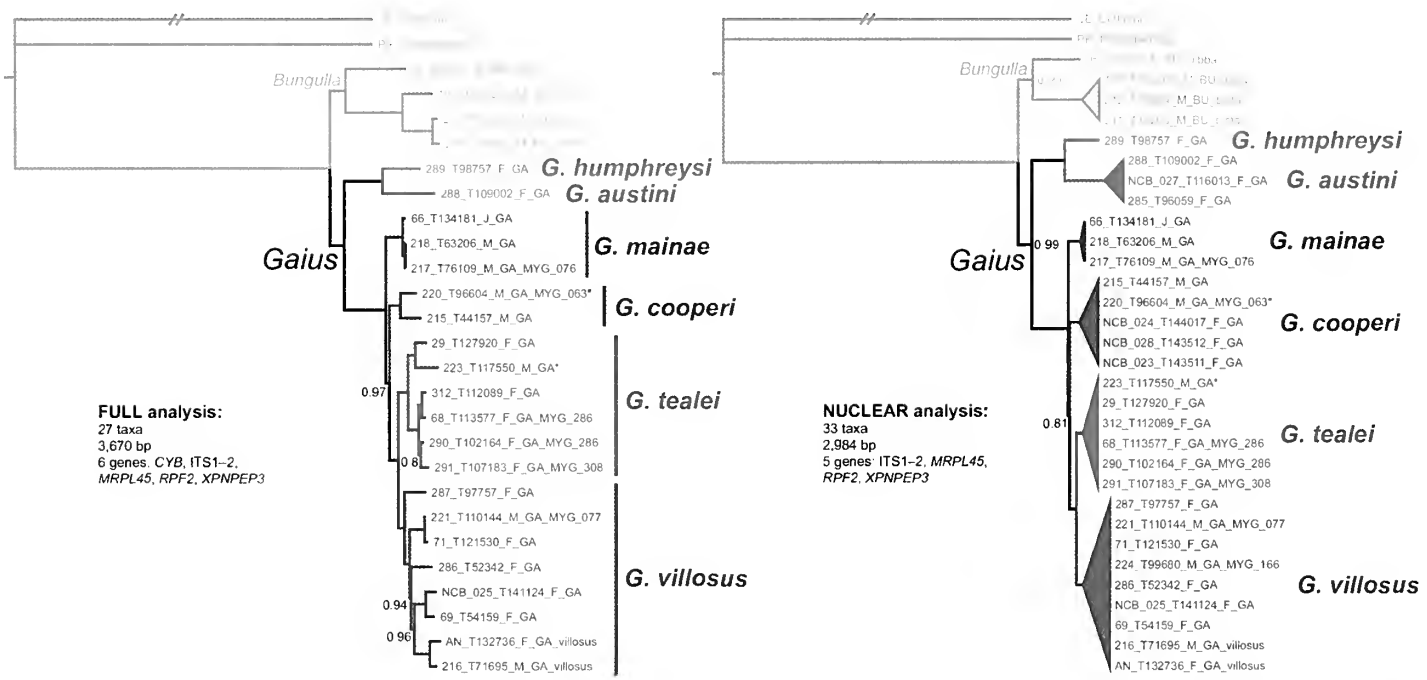


Figure 13.—Bayesian 50% majority-rule consensus trees resulting from partitioned phylogenetic analyses of the 6 gene 'FULL' dataset (at left) and the 5 gene 'NUCLEAR' dataset (at right, with species clades collapsed), color-coded as per Figure 11 and Supplementary File 5, online at http://dx.doi.org/10.1636/JoA-S-17-079.s5. Posterior probabilities < 1.0 are shown adjacent to nodes (all other nodes have a posterior probability of 1.0), and holotype specimens are highlighted (*). Note that sequences were not available for *G. aurora* sp. nov. or *G. hueyi* sp. nov.

RESULTS AND DISCUSSION

Bayesian analyses of the 'FULL' dataset (1 mitochondrial gene, 5 nuclear genes; 3,670 bp) and the 'NUCLEAR' dataset (5 nuclear genes; 2,984 bp) recovered congruent topologies (Fig. 13). Six of the eight species of *Gaius* were sampled for the molecular analyses, and all of these species-group taxa were recovered as well supported monophyletic lineages. *Gaius austini* sp. nov. and *G. humphreysi* sp. nov. consistently formed a deeply divergent clade sister to all other species, and *G. villosus* and *G. tealei* sp. nov. were recovered as sister-species, both results consistent with morphology. Analysis of the expanded 'COI' dataset from Castalanelli et al. (2014) (Supplementary File 5, http://dx.doi.org/10.1636/JoA-S-17-079.s5) also clarified the identification of previously published barcodes, and revealed that *G. tealei* sp. nov. is widespread in the southern-central Pilbara.

While strong mitochondrial phylogeographic structure is evident among populations of *G. tealei* in the Pilbara bioregion (Supplementary File 5, http://dx.doi.org/10.1636/JoA-S-17-079.s5), the deeper phylogeny of *Gaius* is generally characterized by shallow branch lengths (Fig. 13), consistent with late Miocene speciation (as inferred by Rix et al. 2017b). *Gaius* is therefore another example of an aganippine genus which – like *Bungulla* (see Rix et al. 2018b) – has 'broken out' of the semi-arid zone of south-western Australia relatively recently, and is now distributed up to, but not beyond, the Pilbara bioregion. This hypothesis of 'derived xeric adaptation' (see Rix et al. 2015) posits that arid zone taxa are phylogenetically derived relative to their temperate congeners – as *G. villosus* and *G. tealei* sp. nov. are relative to *G. cooperi* sp. nov. and *G. mainae* sp. nov. Unfortunately, sequenceable tissues of *G. hueyi* and *G. aurora* were not available for this study, and future research should focus on sampling and surveying for these rare taxa, both of which may be of conservation significance (see Conservation assessments, below).

SYSTEMATICS

Family Idiopidae Simon, 1889
Subfamily Arbanitinae Simon, 1903
Tribe Aganippini Simon, 1903
Genus *Gaius* Rainbow, 1914

Gaius Rainbow, 1914: 195. Removed from synonymy of *Anidiops* Pocock, 1897 by Rix et al., 2017d: 618 (*contra* Main, 1957: 424).

Type species.—*Gaius villosus* Rainbow, 1914, by monotypy.

Diagnosis.—Species of *Gaius* can be distinguished from all other Aganippini by the densely setose body and appendages (especially the lateral margins of the carapace and the ventral palpal tibia of males) (Fig. 10), by the presence in all but two species (i.e., *G. austini* sp. nov. and *G. humphreysi* sp. nov.) of a very strongly developed distal retrolateral tibial apophysis (dRTA) (Fig. 10), and by the presence in all but three species (i.e., *G. aurora* sp. nov., *G. austini* sp. nov. and *G. humphreysi* sp. nov.) of a massive RTA (Fig. 10) (see also Rix et al. 2017d). Females of *Gaius* can be distinguished from most other Aganippini by their large size and highly setose body and appendages, and by the absence of sclerotized sigilla on the dorsal abdomen (Figs. 1, 2, 4, 5).

Description.—Large to very large idiopid spiders (female body length ca. 30–42 mm; males ca. 20–35 mm), usually dark chocolate-brown to black in life (Figs. 1–5). Carapace oval to almost hexagonal in some species; lateral margins with dense fringe of setae; fovea procurved. Eye group trapezoidal, anterior eye row strongly procurved; ALE relatively widely spaced, usually separated by dense cluster of filiform setae in males. Chelicerae with rastellum; maxillae with cuspules confined to inner corner; labial cuspules absent. Abdomen oval, densely setose; sclerotized sigilla absent. Legs of both sexes with scopulae on tarsi I–II and also on metatarsi I–II of females and some males; male tibia 1 usually with prolateral clasping spurs. Male pedipalp with RTA, in most species forming a massive spinulate process which extends to near retro-distal tip of cymbium; short to very large distal retrolateral tibial apophysis (dRTA) also present; cymbium with small to large field of spinules disto-dorsally. Female pedipalp with thick scopula on tarsus. Female genitalia with pair of simple, widely spaced spermathecae.

Distribution.—The genus *Gaius* is endemic to Western Australia, with a broad distribution that extends largely east of the Jarrah Forest and Geraldton Sandplains bioregions, from Albany, Munglinup, Esperance and Point Dempster in the south, north to Carnarvon and the Fortescue River (Pilbara bioregion) in the north, and east to the eastern margins of the Gascoyne, Murchison, Coolgardie and Mallee bioregions (Fig. 11). Isolated, outlying populations also occur in the more mesic Jarrah Forest bioregion, at Collie and Voyager Quarry, the latter of which may now be extinct (see Remarks under *G. cooperi* sp. nov., below).

Habitat, natural history and phenology.—Thanks to the demographic work undertaken by Barbara Main at North Bungulla Nature Reserve over more than 40 years, we now have a very good understanding of the biology and natural history of *G. villosus* in the central Wheatbelt (Main 1978, 1987, unpubl. data). Being very large and conspicuous (Fig. 3), male *Gaius* are also routinely submitted to the WAM by members of the public, providing a data-rich phenology series for a number of species, and for the genus as a whole. *Gaius* are most common in the mallee and mulga woodlands, *Acacia* shrublands and spinifex (*Triodia*) plains of the Western Australian interior, where they build deep burrows in clay or hard loam soils. Burrow doors are flappy or wafer-like, and burrow entrances are typically adorned with a characteristic radial 'fan' of twig-lines (Figs. 6–9). Burrows are also usually lined with a sock-like silken 'collar' several centimeters below the burrow entrance (see Main 1985, figs. 209, 210). When disturbed, this silken collar can be collapsed and pulled downwards by the spider, creating a physical barrier to protect the inhabitant against predators and flooding (Main 1957, 1985, 1993). Food debris is also stored between this collar and the wall of the burrow proper, and prey consists largely of ants and termites (Main 1978).

Male spiders (at least of *G. villosus*) usually mature around seven or eight years of age (Main 1987), and phenology data for the genus as a whole ($n = 183$) shows that males generally wander in search of females in the warm summer months of November–March (88% of collected specimens), usually after heavy thunderstorms, with nearly half of all specimens (44%) collected in December or January (Fig. 12). If these storms do not occur, then males of *G. villosus* wander with the first

autumn/winter rains (Main 1987); no male specimens of *Gaius* have ever been collected in September. This strategy of summer emergence of males is in stark contrast to most other Idiopidae Australia-wide, which generally emerge in the cooler months from late autumn to early spring (e.g., Main et al. 2000; MGR, RJR & MSH, pers. obs.).

Female spiders (at least of *G. villosus*) mature around eight years of age, but do not reproduce until they are older (Main 1987). Eggs are laid in spring, and prior to egg-laying burrows are plugged with a thick mud barrier beneath the door, thus completely sealing in the mother spider and egg sac (and later spiderlings) during the hot summer and early autumn months (Main 1978). These burrow plugs are not removed until the following autumn, prior to the emergence of the spiderlings with seasonal autumn or winter rains. Females can reproduce at most once every two years (Main 1987), probably due to the energetic requirements of reproduction and maternal aestivation during the summer months when males wander in search of females. However, it is not known whether un-molted females can store sperm between successive broods, or whether a new mating is required prior to egg-laying.

The longevity of *G. villosus* is well documented, and this species is undoubtedly one of the longest-lived spiders in the world, with some specimens persisting for over 40 years in the wild (Main 1987, unpubl. data). It is therefore likely that large, reproductive 'matriarchs' of all *Gaius* species routinely live to over 20 years of age.

Composition and remarks.—*Gaius* was found to be the sister-genus to *Eucyrtops* by Rix et al. (2017b; Fig. 10), and includes eight known species, seven of which are newly described in this study. All species are large to very large, highly setose and dark brown to black in color (Figs. 1–5), and the genus includes some of the largest mygalomorph spiders in Australia, excluding Theraphosidae and perhaps some specimens of *Hadronyche formidabilis* (Rainbow, 1914) (Atracidae), *Xamiatus* Raven, 1981 (Nemesiidae) and *Idiosoma* (Idiopidae). Unlike the co-occurring and highly diverse aganippine genera *Bungulla* and *Idiosoma*, species of *Gaius* are largely absent from Western Australia's coastal and inland sandplains, and are conspicuously absent from the Swan Coastal Plain, Geraldton Sandplains and much of the southern Carnarvon Basin (Fig. 11).

KEY TO THE AUSTRALIAN SPECIES OF *GAIUS* (MALES ONLY)

NB. See also Supplementary File 1, online at http://dx.doi.org/10.1636/JoA-S-17-079.s1, for additional images of relevant character states.

1. Prolateral tibia 1 with clasping spurs (Figs. 23, 57, 79, 101, 114, 154) 2
– Prolateral tibia 1 without clasping spurs, replaced by comb of distal macrosetae (Figs. 45, 133) 7
2. Palpal tibia with massive RTA, extending to near retro-distal tip of cymbium (Figs. 25, 81, 103, 156) 3
– RTA much smaller, without grossly enlarged morphology (Figs. 59, 116) 6
3. Palpal tibia proximal to RTA short and stout, with bulging, convex ventral margin in retrolateral view (Figs. 25, 156); leg I tibia relatively long, with clasping spurs occupying distal quarter of tibia (Figs. 22, 153) 4
– Palpal tibia proximal to RTA longer and less bulging in retrolateral view (Figs. 81, 103); leg I tibia shorter, with clasping spurs occupying distal third of tibia (Figs. 78, 100) 5
4. Distal retrolateral tibial apophysis (dRTA) very large, 'ladle-shaped' (Fig. 25); base of dRTA separated from base of RTA by crescent-shaped retro-ventral margin of palpal tibia (Fig. 25); pro-ventral tarsus I usually with row of prominent, stout macrosetae protruding from surrounding scopulate setae (Fig. 22) ..... ***G. villosus*** Rainbow, 1914
– dRTA much shorter, 'tongue-shaped' (Fig. 156); base of proximal flange of dRTA positioned closely adjacent to base of RTA (Fig. 156); pro-ventral tarsus I without row of protruding macrosetae (Fig. 153) ..... ***G. tealei*** sp. nov.
5. dRTA hooked, with proximal 'stem' and variably-shaped, distally widened 'knob' (Figs. 81, 82) ..... ***G. cooperi*** sp. nov.
– dRTA not hooked, forming simple, rounded, disto-ventrally directed process (Figs. 103, 104) ..... ***G. hueyi*** sp. nov.
6. ALE separated by approximately their own diameter (Fig. 109); dRTA broad, subquadrate in retrolateral view (Fig. 116); embolic apophysis positioned distally (Figs. 116, 118) ..... ***G. huuphreysi*** sp. nov.
– ALE separated by approximately twice their own diameter (Fig. 52); dRTA slightly longer and more tapered in retrolateral view (Fig. 59); embolic apophysis positioned sub-distally (Figs. 59–61) ..... ***G. austini*** sp. nov.
7. Palpal tibia with very large RTA, extending to near retro-distal tip of cymbium (Fig. 134) ..... ***G. mainae*** sp. nov.
– RTA much smaller (Fig. 46) ..... ***G. aurora*** sp. nov.

Gaius villosus Rainbow, 1914
(Figs. 1–3, 6–8, 10, 11, 14–36)

Gaius villosus Rainbow, 1914: 195, figs. 6–8.

Anidiops villosus (Rainbow): Main, 1957: 426, figs. 3C, 5B; Main, 1985: 16, figs. 20, 21, 192.

Gaius villosus Rainbow: Rix et al., 2017d: 620, figs. 238, 239, 242, 246, 248, 252, 253.

Gaius sp. 'Wiluna' Rix et al., 2017d: 619, figs. 244, 251.

Type material.—*Holotype female.* AUSTRALIA: *Western Australia*: Minnivale (IBRA_AVW), 31°08′S, 117°11′E, hand collected, 31 March 1913, J.P. Harris (AMS KS6259; not examined).

Paratype. AUSTRALIA: *Western Australia*: 1 ♀, same data as holotype except 1 March 1913 (WAM T261; examined).

Other material examined.—AUSTRALIA: *Western Australia*: 1 ♂, Minnivale (IBRA_AVW), 31°08′S, 117°11′E, 1 March 1976, L. & T. Cooke (WAM T27036); 1 ♂, same data except lot 10 Dowell Street, 20 March 1999, R.J. Larkin (WAM T41700); 1 ♂, same locality data except Mardu, 1999 (WAM T143034); 1 ♀, Minnivale Nature Reserve, 19 km WNW. of

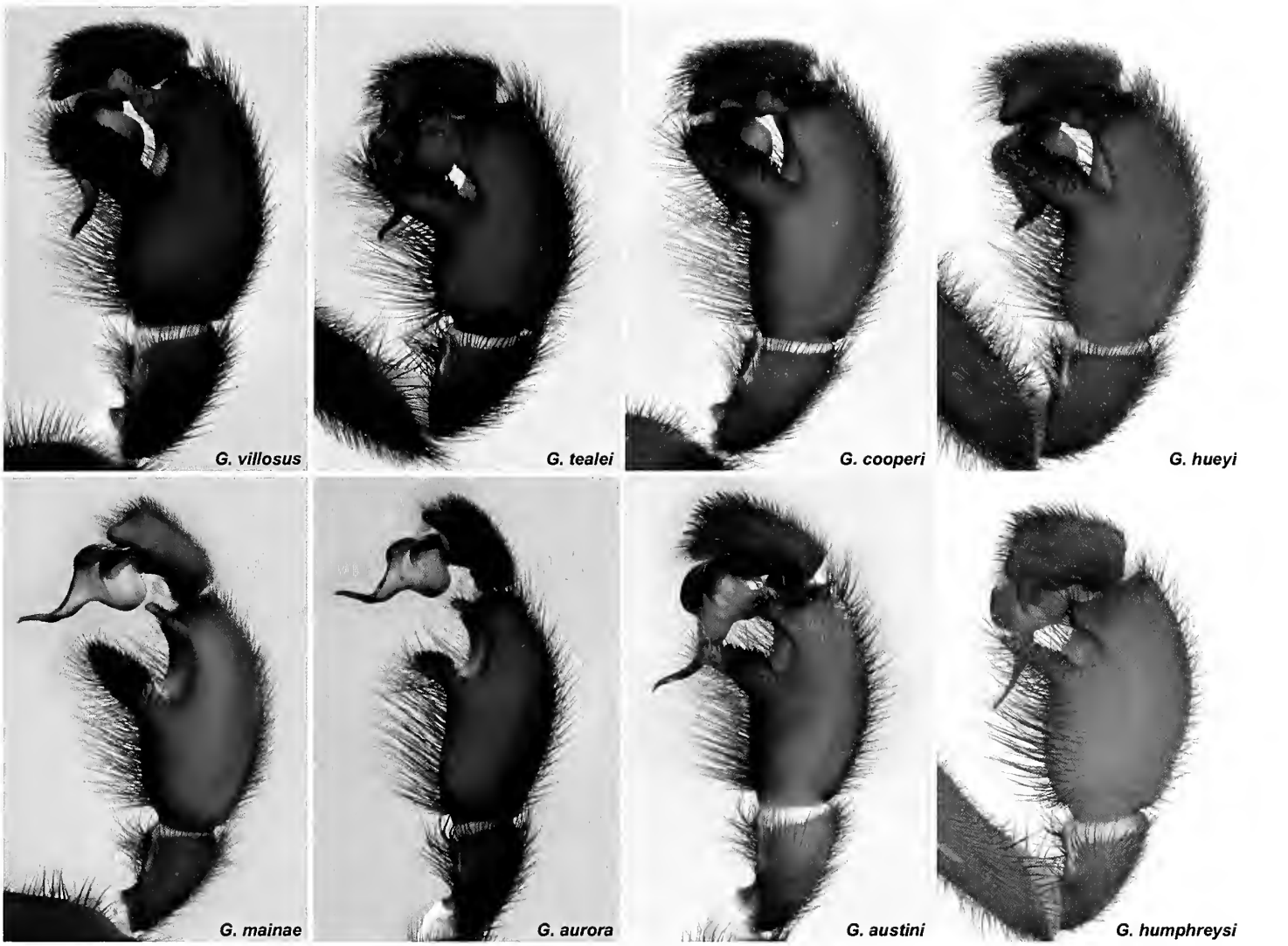
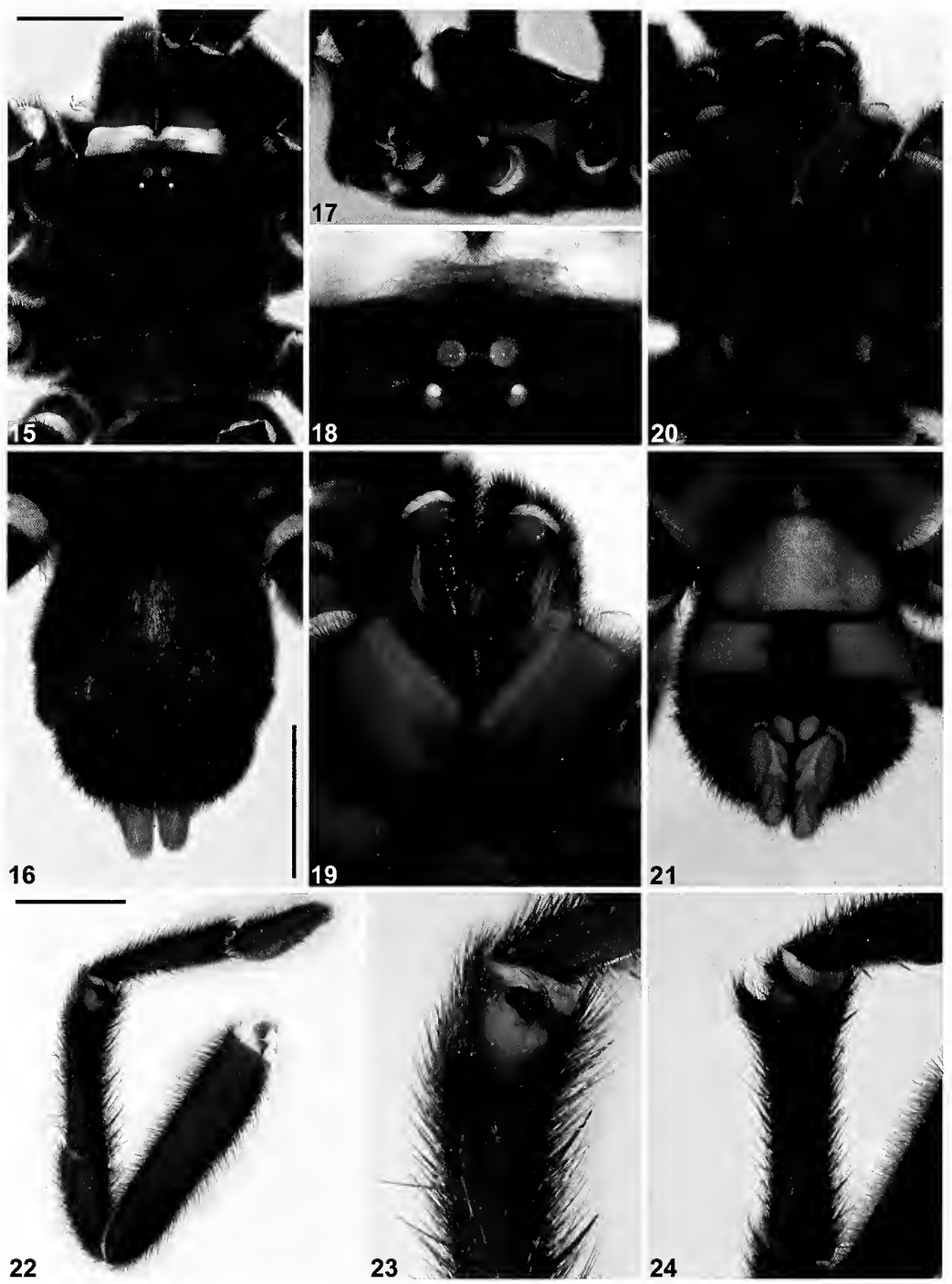


Figure 14.—Montage of male pedipalps of species of *Gaius*. Note the variation in the size and shape of the distal retrolateral tibial apophysis (dRTA).

Wyalkatchem (IBRA_AVW), 31°08′11″S, 117°11′22″E, hand collected, mallee woodland, 21 April 2014, M.G. Rix, M.S. Harvey (WAM T132736[DNA_Voucher_AN]; GenB–COI–KY295234, GenB–CYB–KY295359, GenB–MRPL45–KY295484, GenB–RPF2–KY295600, GenB–XPNPEP3–KY295728, GenB–ITS–KY294978); 1 ♂, Agnew Mining Camp (IBRA_MUR), 27°49′S, 120°41′E, 1 November 1977, A. Schofield (WAM T26996); 1 ♂, Banksia Patch East, c. 13.5 km N. of Kellerberrin on Trayning Road (Colin Wilkins, CSIRO banksia remnant 16) (IBRA_AVW), 31°31′S, 117°44′E, drowned in frog trap, 19 April 2002, R. Davis (WAM T143041); 1 ♂, Beacon townsite (IBRA_AVW), 30°26′S, 117°52′E, hand collected, January 2003, B.R. Kirby (WAM T47876); 1 ♂, Big Bell (IBRA_MUR), 27°19′S, 117°39′E, 16 January 1990, T. Baker (WAM T20548); 1 ♂, Billabong Roadhouse (IBRA_CAR), 26°49′S, 114°37′E, hand collected, 9 December 1994, C. Quinn (WAM T32193); 1 ♂, Blue Hill Range (IBRA_YAL), 29°08′38″S, 116°53′40″E, dry pitfall trap, ironstone ridge in mulga/eucalypt woodland, 13–16 February 2004, M.J. Bamford (WAM T57384); 1 ♂, Bonnie Rock (IBRA_AVW), 30°32′S, 118°22′E, 11 January 1993, G. Borlalse (WAM T26998); 1 ♂, Bulong (IBRA_COO), 30°45′S, 121°48′E, 29 January 1937, M. Jones (WAM T2965); 1 ♂, Buntine (IBRA_AVW), 29°59′S, 116°34′E, 4 February 1935, L.D. Manuel (WAM T2751); 1 ♂, same data except 20 November 1982, K. Wilkin (WAM T27000); 1 ♂, Carnamah (IBRA_AVW), 29°41′25″S, 115°53′01″E, 1 February 1985, S. Forrester (WAM T 21059); 1 ♂, Carnarvon (IBRA_CAR), 24°53′S, 113°40′E, 9 July 1949, Mr. Bosworth (WAM T3515); 1 ♂, same data (WAM T3516); 1 ♂, same data except 2 January 1965, L. Craig (WAM T27002); 1 ♂, same data except 1 November 1989 (WAM T27003); 1 ♂, 50 km SE. of Carnarvon (IBRA_CAR), alive on road at night, 22 February 2008, B. Maryan (WAM T88456); Caron (IBRA_AVW), 29°36′S, 116°20′E, hand collected, 1 October 1953, B.H. Kuhne (WAM T3745); 1 ♀, Charles Darwin Reserve, N. of Smith Well (IBRA_AVW), 29°36′33″S, 116°58′04″E, dug from burrow, 8 May 2009, M.S. Harvey, B. Barnett, C. Hodge, C. Richard (WAM T97757[DNA_Voucher_287]; GenB–CYB–MG652505, GenB–MRPL45–MG652541, GenB–RPF2–MG652567, GenB–XPNPEP3–MG652587, GenB–ITS–MG652526); Cogla

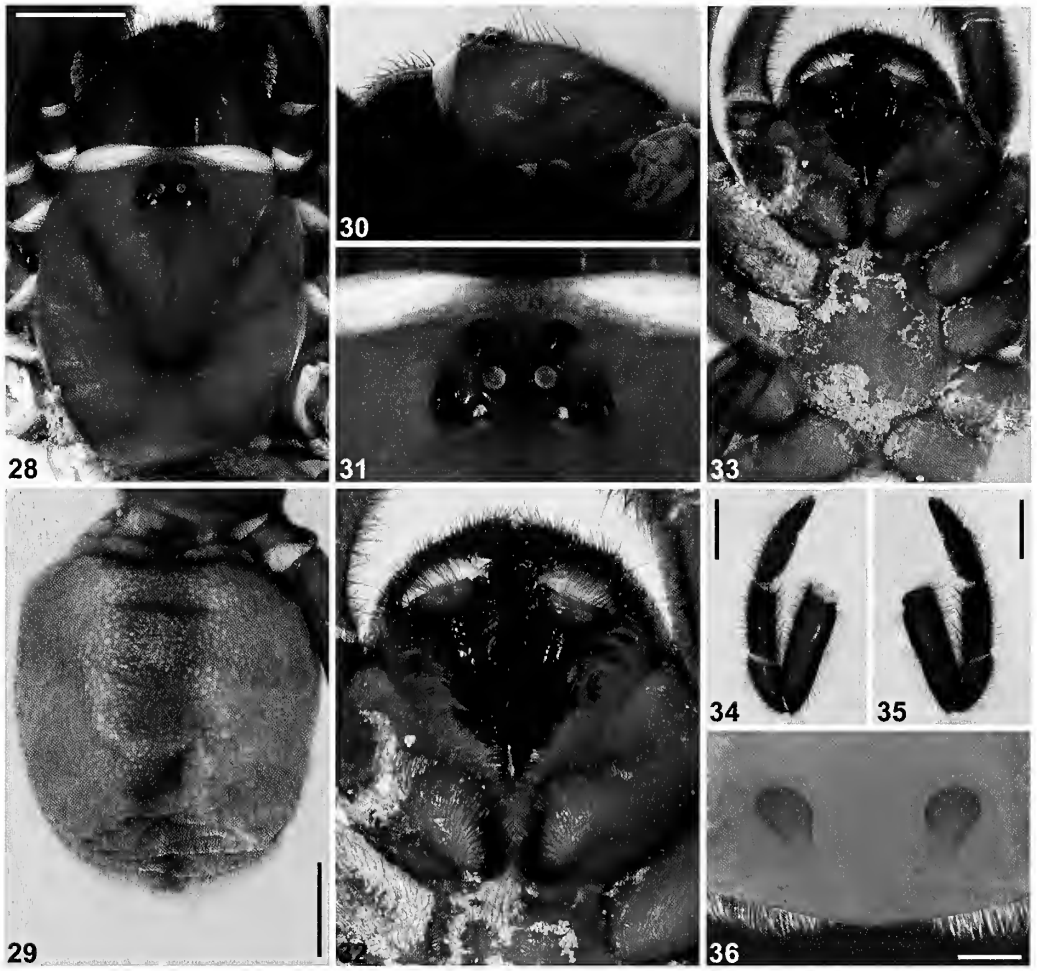


Figures 15–24.—*Gaius villosus* Rainbow, 1914, male (WAM T41700) from Minnivale (Western Australia: AVW), somatic morphology: 15–16, carapace and abdomen, dorsal view; 17, cephalothorax, lateral view; 18, eyes, dorsal view; 19, mouthparts, ventral view; 20–21, cephalothorax and abdomen, ventral view; 22, leg I, prolateral view; 23, leg I tibia, clasping spurs, prolateral view; 24, leg I tibia, proventral view. Scale bars = 5.0.

Downs Station, 70 miles NNW. of Sandstone (IBRA_MUR), 27°26′S, 118°56′E, 1 January 1981, A.R. Humphries (WAM T27004); 1 ♂, Deception Hill, 112.71 km NNW. of Koolyanobbing (IBRA_COO), 29°50′59″S, 119°16′58″E, dry pitfall trap, 7 December 2010, R. Teale, Z. Hamilton, V. Cartledge (WAM T110144[DNA_Voucher_221]; GenB–CYB–MG652504, GenB–MRPL45–MG652539, GenB–RPF2–MG652559, GenB–XPNPEP3–MG652576, GenB–ITS–MG652521); Dowerin (IBRA_AVW), 31°12′S, 117°02′E, hand collected, 26 January 1940, D. Jones (WAM T3208); 1 ♂, same data except 1 January 1948, P. O'Shaughnessy (WAM T3479); 1 ♂, same data except 12 January 1981, P. Emmott (WAM T27012); 1 ♂, same data except indoor basketball courts, 28 February 1997, J. Pickering, N. Northey (WAM T41701); 1 ♂, 25 miles NE. of Dowerin (IBRA_AVW), 30°57′S, 117°20′E, 22 January 1973, L.F. Bear (WAM

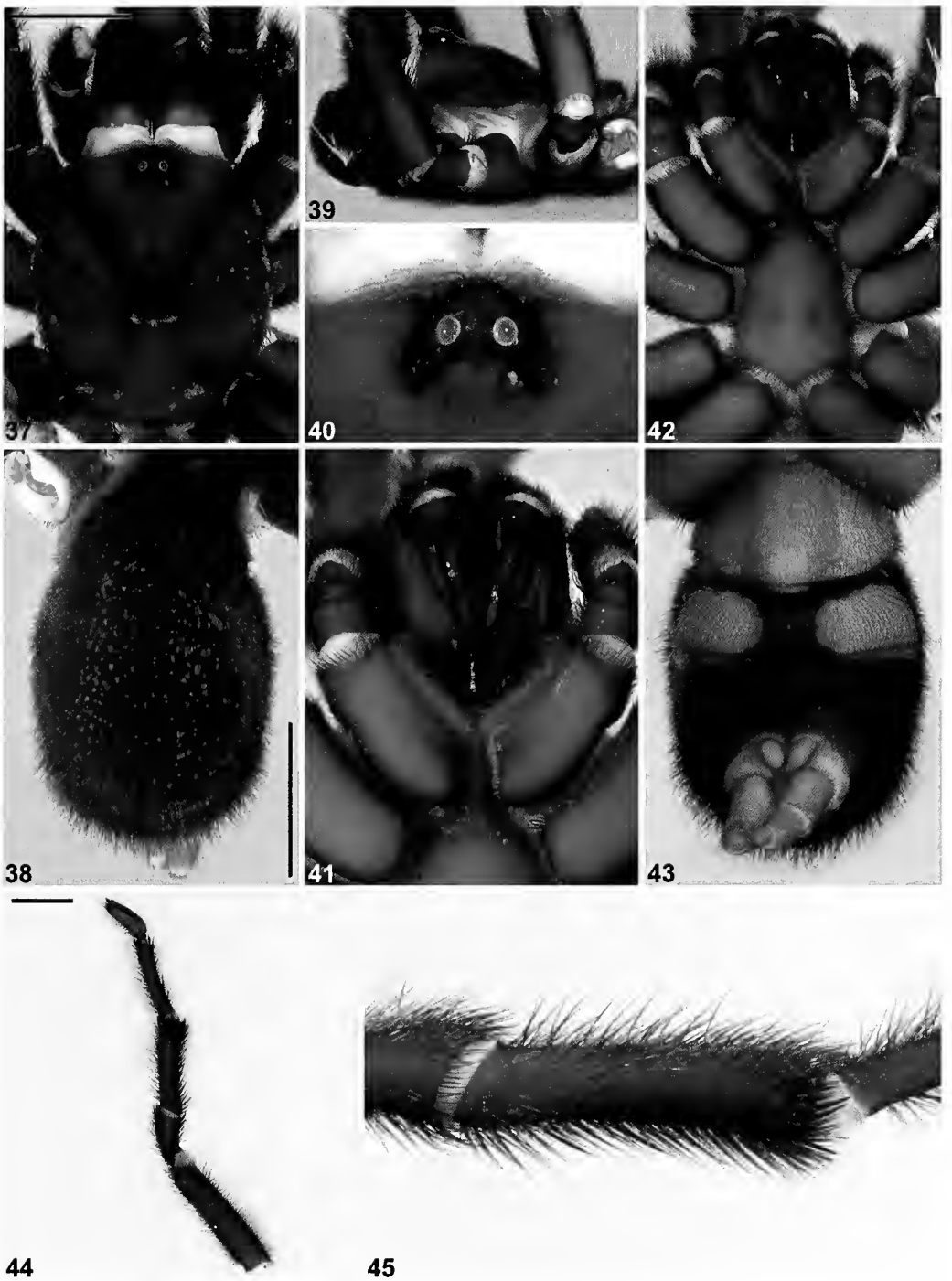
Figures 25–27.—*Gaius villosus* Rainbow, 1914, male (WAM T41700) from Minnivale (Western Australia; AVW), pedipalp: 25, retrolateral view (arrow to embolic apophysis); 26, retroventral view; 27, prolateral view. Scale bar = 5.0.

T27011); 1 ♂, Durokoppin Nature Reserve, site B4 (IBRA_AVW), 31°30′S, 117°44′E, 6–16 January 1989, G. Friend et al. (WAM T63225); 1 ♂, same data except site DKR G2, wet pitfall trap (WAM T41518); 1 ♂, Elashgin Nature Reserve, N. side on Maitland Road (IBRA_AVW), 31°19′35″S, 117°26′41″E, wet pitfall traps, 29 November 1999–17 March 2000, M.S. Harvey, J.M. Waldock, B.Y. Main (WAM T44162); 1 ♂, Gnaweeda Siding via Meekatharra (IBRA_MUR), 26°35′S, 118°44′E, hand collected, 7 November 1938, W. Doubtfire (WAM T3098); 1 ♂, corner of Great Northern Highway and Mount Gibson Road (IBRA_AVW), 29°38′30″S, 117°07′56″E, 21 November 2010, R. Ellis (WAM T109379); 1 ♂, Hamelin Pool, Telegraph Station (IBRA_CAR), 26°24′S, 114°10′E, 1 May 1994, D. Taylor (WAM T31062); 1 ♂, Kalannie (IBRA_AVW), 30°22′S, 117°07′E, found at night on house doorstep, 3 April 1987, M. Tierney (WAM T27019); 1 ♂, same locality data, 17 February 1996, B. & J. Davies (WAM T42167); 1 ♀, ca. 154 km NE. of Kalbarri, N. of Toolonga Nature Reserve (IBRA_CAR), 26°37′19″S, 115°11′56″E, from pipeline trench, 27 October 2007, P. Hinchy (WAM T54159^{DNA_Voucher_69}; GenB–CYB–KY295431, GenB–MRPL45–KY295551, GenB–RPF2–KY295677, GenB–XPNPEP3–KY295805, GenB–ITS–KY295058); 1 ♂, Kellerberrin (IBRA_AVW), 31°38′S, 117°43′E, 1 January 1992, A.F. & J.L. Morley (WAM T27024); 1 ♂, Koorda (IBRA_AVW), 30°50′S, 117°29′E, 15 January 1935, V.J. Geraghty (WAM T2741); 1 ♂, same locality data, 2 November 1949 (WAM T3551); 1 ♂, same data except bushland around house, 28 January 1991, L. Henning (WAM T22530); 1 ♂, same locality data, 16 November 1972, S. Cornish (WAM T27025); 1 ♂, same data except 11 January 1973, C.D. Strahan (WAM T27026); 1 ♂, same data except 15 December 1978, B. Kennewell (WAM T27027); 1 ♂, same data except June 1954, L. Glauert (WAM T143042); 1 ♂, Korrelocking (IBRA_AVW), 31°12′S, 117°28′E, 10 December 1951, J.C. Pearson (WAM T3659); 1 ♂, Lake Goorly, north-west, site WU 8 (IBRA_AVW), 29°56′08″S, 116°53′09″E, wet pitfall traps, 15 September 1998–25 October 1999, P. Van Heurck, CALM Survey (WAM T143043); 1 ♂, Lake Maitland mine lease, ca. 22 km SW. of Wonganoo Homestead, site LM01 (IBRA_MUR), 27°12′54.8″S, 121°07′57.1″E, dry pitfall trap, 12 December 2007, P. Bolton et al. (WAM T136897); 2 ♂, Lake Mason, near Sandstone (IBRA_MUR), 27°41′17.3″S, 119°18′15.5″E, dry pitfall trap, 3 December 2008, S. Tomlinson (WAM T143060); 1 ♂, same data (WAM T136899); 1 ♂, Latham (IBRA_AVW), 29°45′28″S, 116°26′36″E, 1 January 1976, P. Bercene (WAM T27029); 1 ♂, same data (WAM T27030); 1 ♂, Leinster (IBRA_MUR), 27°55′S, 120°41′E, 21 December 1994, D. Murphy (WAM T143062); 1 ♂, 27 km SE. of Leinster Downs Homestead (IBRA_MUR), 28°01′S, 120°48′E, 5 November 1999, G. Harold (WAM T40232); 1 ♂, Leonora (IBRA_MUR), 28°53′S, 121°20′E, 17 January 1965, A.J. Fox (WAM T27032); 1 ♂, same data except 1 January 1987, C.N. Collard (WAM T27033); 1 ♂, same locality data, 21 December 1999 (WAM T41549); 1 ♂, NW. of Leonora (IBRA_MUR), 28°53′S, 121°20′E, 1 December 1988, S. Gilligan (WAM T27031); 1 ♂, Lorna Glen Station, quadrat 21 (IBRA_GAS), 26°04′09″S, 121°26′54″E, dry pitfall trap, 25 November–1 December 2004, M.A. Cowan et al. (WAM T66406); 2 ♂, same data except quadrat 23, 26°06′46″S, 121°30′15″E (WAM T66409); 1 ♂, Manmanning (IBRA_AVW), 30°51′S, 117°06′E, 31 March 1960, B.H. Smith (WAM T27034); 1 ♂, same data (WAM T27035); 1 ♂, Manmanning town reserve (IBRA_AVW), 30°51′14″S, 117°05′48″E, wet pitfall trap, 14 January–21 April 1997,



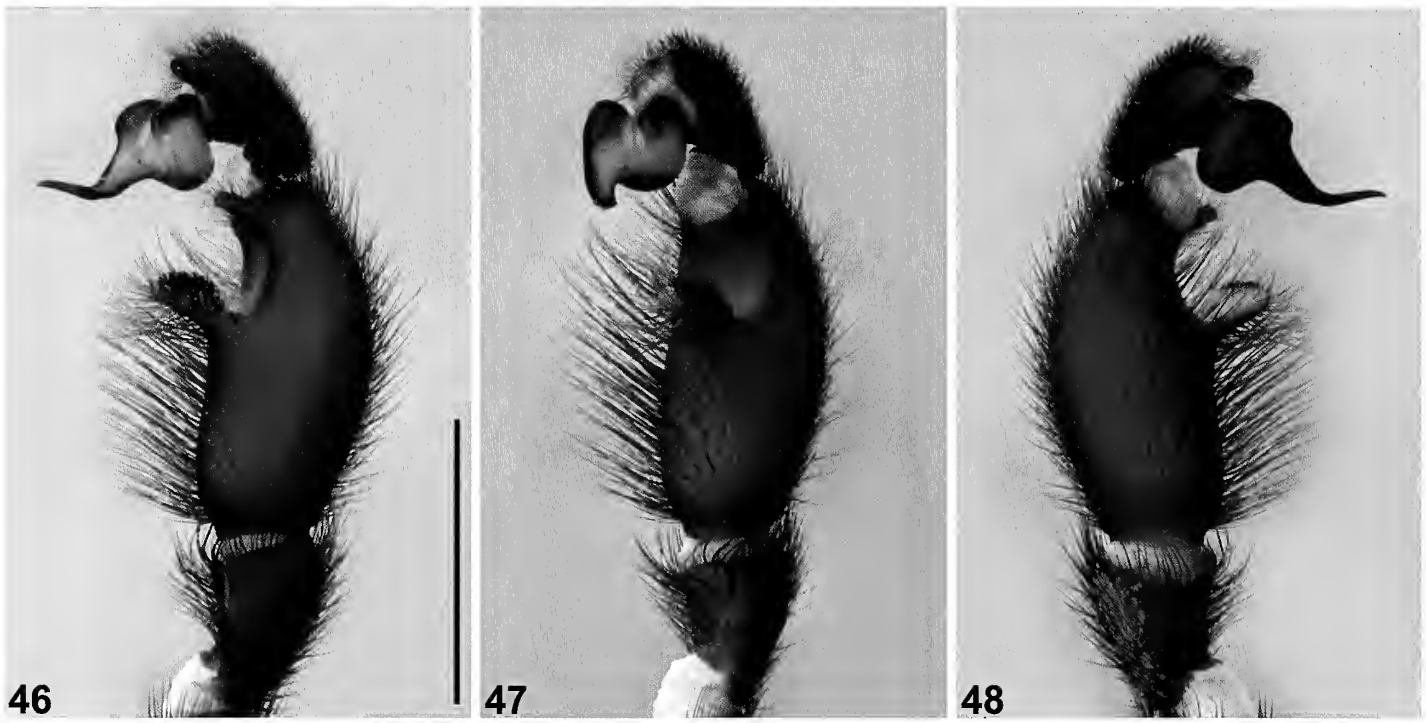
Figures 28–36.—*Gaius villosus* Rainbow, 1914, female (WAM T132736) from Minnivale Nature Reserve (Western Australia; AVW): 28–29, carapace and abdomen, dorsal view; 30, cephalothorax, lateral view; 31, eyes, dorsal view; 32, mouthparts, ventral view (partly obscured by dried hemolymph); 33, cephalothorax, ventral view (partly obscured by dried hemolymph); 34, leg I, prolateral view; 35, leg I, retrolateral view; 36, spermathecae, dorsal view. Scale bars = 5.0 (28–29, 34–35), 1.0 (36).

J.M. Waldock, E.S. Volschenk (WAM T40233); Manmanning Dam Nature Reserve, south-east, site WH 8 (IBRA_AVW), 30°54′53″S, 117°05′41″E, wet pitfall traps, 15 September 1998–25 October 1999, L. King, CALM Survey (WAM T143038); 1 ♂, Maya (IBRA_AVW), 29°53′S, 116°30′E, hand collected, 27 December 1999, C. McLevie (WAM T40230); 1 ♂, ca. 130 km N. of Meekatharra, site 2B-P6 (IBRA_GAS), 25°34′37.84″S, 118°54′37.83″E, pitfall trap, 12 November 2009, M. Peterson (WAM T99680[DNA_Voucher_224]; GenB–CO1–KJ745514, GenB–MRPL45–MG652540, GenB–RPF2–MG652560, GenB–XPNPEP3–MG652575, GenB–ITS–MG652527); 1 ♂, same data except site 5B-P6, 25°38′30.28″S, 119°04′50.02″E, 14 November 2009, K. George (WAM T99681); 1 ♂, Meeline Station, Mount Magnet (IBRA_MUR), 28°27′S, 118°16′E, 7 January 2001, K. Morrisey (WAM T143063); 2 ♂, same data except 3 October 1999 (WAM T143065); 1 ♂, Milly Milly Station (IBRA_MUR), 26°04′S, 116°41′E, 1 December 1992, M. Broad (WAM T26994); 1 ♂, Moonijin, 14 miles N. of Cadoux (IBRA_AVW), 30°57′S, 117°05′E, 12 December 1969, J.L. Emmott (WAM T27039); 1 ♂, same data except 28 November 1987, P.L. Emmott (WAM T27038); 1 ♂, same data except 18 December 1979, R.H. Harvey (WAM T27037); 1 ♂, Mount Burges Station (IBRA_COO), 30°50′S, 121°06′E, 1 March 1988, Warburton (WAM T27042); 1 ♂, Mount Gibson area (IBRA_AVW), pitfall trap, February 2008, B. Maryan (WAM T88457); 1 ♂, Mount Gibson mining lease, site E4 (IBRA_AVW), 29°35′17.64″S, 117°12′04.14″E, dry pitfall trap, eucalypt woodland, 6 February 2008, S. Thompson et al. (WAM T86669); 2 ♂, same data except site E6 + 8, 29°34′S, 117°11′E (WAM T86675); 1 ♀, Mount Ida, 80 km NW. of Menzies (IBRA_MUR), 29°14′04″S, 120°23′44″E, hand foraging, *Acacia* shrubland, 4 October 2011, V. Saffer (WAM T121530[DNA_Voucher_71]; GenB–CYB–KY295429, GenB–MRPL45–KY295549, GenB–RPF2–KY295675, GenB–XPNPEP3–KY295803, GenB–ITS–KY295056); 1 ♂, Mount Magnet (IBRA_MUR), 28°04′S, 117°51′E, 1 January 1986, Thompson (WAM T27043); 2 ♂, Mount Vetters Station, Black Swan Nickel Mine, 50 km NE. of Kalgoorlie, control 5 (IBRA_MUR), 30°23′29″S, 121°29′10″E, wet pitfall trap, 11 December 2003–5 January 2004, P.R. Langlands (WAM T53311); 1 ♂, Muckinbudin (IBRA_AVW), 30°55′S, 118°12′E, 17 November 2001, H. Adamson (WAM T143040); 1 ♂, Mullewa (IBRA_AVW), 28°32′S, 115°29′E, in house, August 1966, S.R. White, J. O'Brien (WAM T143039); 1 ♂,



Figures 37–45.—*Gaius aurora* sp. nov., male holotype (WAM T16076) from Bungalbin Hill, Helena-Aurora Range (Western Australia: COO), somatic morphology: 37–38, carapace and abdomen, dorsal view; 39, cephalothorax, lateral view; 40, eyes, dorsal view; 41, mouthparts, ventral view; 42–43, cephalothorax and abdomen, ventral view; 44, leg I, prolateral view; 45, leg I tibia, prolateral view. Scale bars = 5.0.

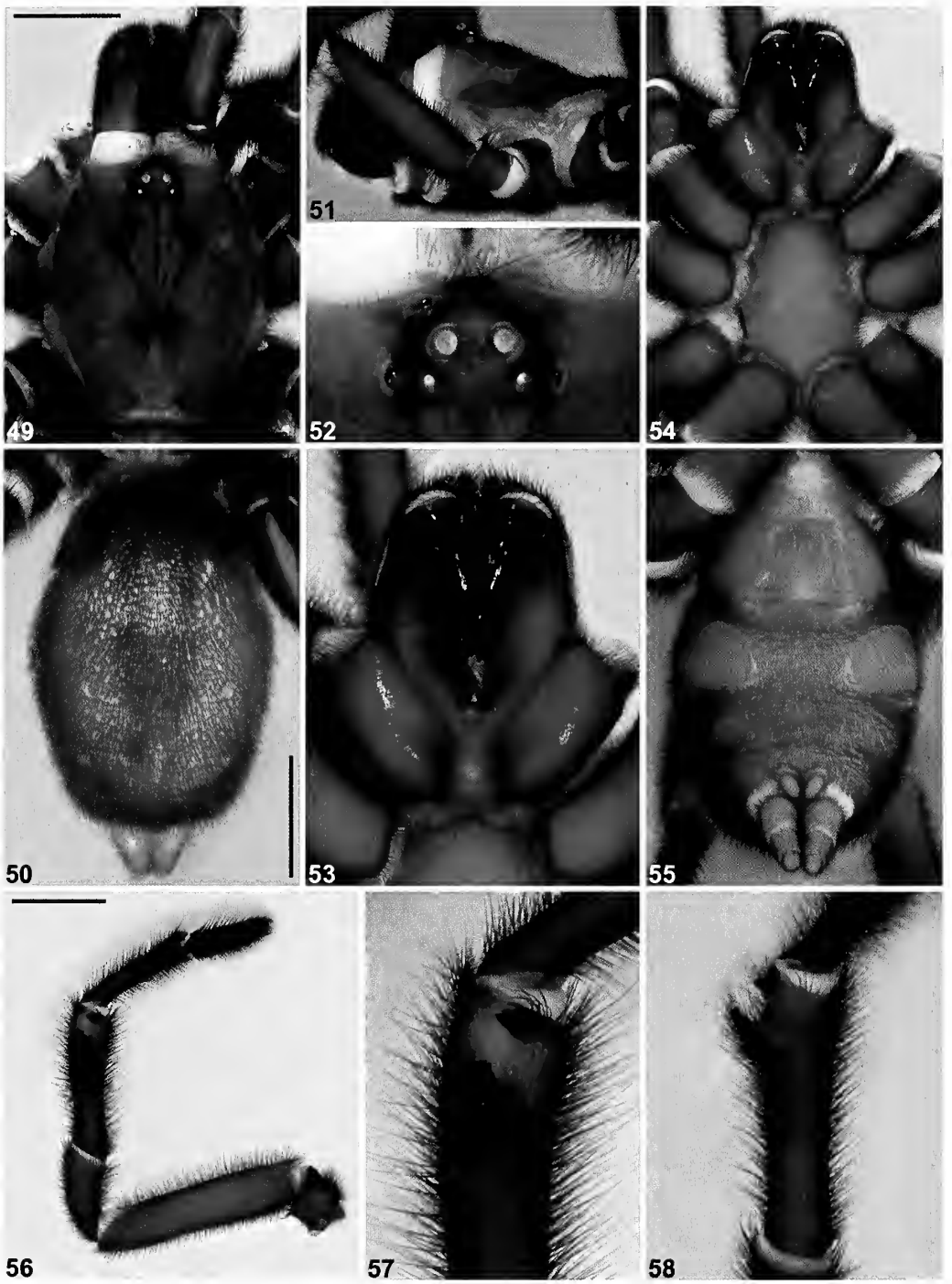
Nerren Nerren Station, site NE1 (IBRA_YAL), 27°03′23.6″S, 114°35′21.3″E, wet pitfall trap, 16 October 1994–11 January 1995, N.L. McKenzie, J. Rolfe (WAM T48301); 1 ♂, North Baandee (IBRA_AVW), 31°22′S, 117°56′E, 3 February 1985, M. Enright (WAM T21070); 1 ♂, North Cleary, Shire of Mount Marshall (IBRA_AVW), 30°26′S, 117°39′E, 1 February 1979, A. Putt (WAM T27047); 1 ♀, junction of North-West Coastal Highway and Vermin Proof Fence (IBRA_YAL), 27°19′00″S, 114°36′11″E, hand collected from burrow, 18 August 2016, M.S. Harvey, M.E. Blosfelds (WAM T141124[DNA_Voucher_NCB_025]; GenB–CYB–MG652499, GenB–MRPL45–MG652537, GenB–RPF2–MG652557, GenB–XPNPEP3–MG652578, GenB–ITS–MG652520); 1 ♂, Nulla Nulla, Yorkrakine (IBRA_AVW), 31°22′S, 117°35′E, 29 November 1965, N. Naughton (WAM T27048); 1 ♂, The Overlander [Road House], Shark Bay Road (IBRA_CAR), 26°25′S, 114°28′E, February 1970 (WAM T143050); 1 ♂, Perenjori (IBRA_AVW), 29°26′35″S, 116°17′07″E, 15 January



Figures 46–48.—*Gaius aurora* sp. nov., male holotype (WAM T16076) from Bungalbin Hill, Helena-Aurora Range (Western Australia; COO), pedipalp: 46, retrolateral view; 47, retroventral view; 48, prolateral view. Scale bar = 5.0.

1993, R. Young (WAM T26995); 1 ♂, same data except 9 February 1974, J. Billinghurst (WAM T27049); 1 ♂, same locality data except 29°26′S, 116°17′E, 25 January 2000, M. Ripper (WAM T40579); 1 ♂, Pinnacles Station (IBRA_MUR), 28°12′S, 120°26′E, 12 November 1987, R. Duncan (WAM T27050); 1 ♂, 28 km SE. of Pinnacles Homestead (IBRA_MUR), 28°23′S, 120°38′E, 3 November 1999, G. Harold (WAM T40234); 1 ♂, Sandstone (IBRA_MUR), 27°59′S, 119°18′E, 1 February 1971, D.B. Ross (WAM T27083); 1 ♀, Tallering Peak (IBRA_YAL), 28°06′S, 115°38′E, 16–19 May 2003, M.J. Bamford (WAM T52342[DNA_Voucher_286]; GenB–CYB–MG652506, GenB–MRPL45–MG652538, GenB–RPF2–MG652558, GenB–XPNPEP3–MG652586, GenB–ITS–MG652528); 1 ♂, Trayning (IBRA_AVW), 31°06′49″S, 117°47′26″E, 16 December 1988, A. Dugand (WAM T27066); 1 ♂, same data except 1 November 1976, R.W. Hawkes (WAM T27145); SSW. of Trayning (lot 340, loc. 11903), SE. of Yelbeni (IBRA_AVW), 10 January 2000, M. Barnes (WAM T40231); 6 ♂, 30 km S. of Wiluna (IBRA_MUR), 26°52′S, 120°41′E, dry pitfall traps and funnels, mulga woodland, 23 October 2006, S. Thompson (WAM T132589); 1 ♂, Winchester (7 miles from Carnamah) (IBRA_AVW), 29°46′S, 115°55′E, 16 December 1981, C. Chapman (WAM T27070); 1 ♂, Wongan Hills (IBRA_AVW), 30°49′S, 116°37′E, 18 December 1978, H. Taylor (WAM T27071); 1 ♂, same data except outside house, 26 December 1991, S. Mallet (WAM T27072); 1 ♂, Wongan Hills townsite (IBRA_AVW), 30°53′S, 116°43′E, found dead in web of *Latrodectus hasselti* Thorell, 1870, 6 July 1995, D. Smith (WAM T45699); 1 ♂, 1.5 km NNW. of Wongan Hills on Waddington-Wongan Hills Road (IBRA_AVW), 30°54′S, 116°43′E, 8 January 2006, D. Algaba (WAM T71695[DNA_Voucher_216]; GenB–COI–KY295248, GenB–CYB–KY295372, GenB–MRPL45–KY295496, GenB–RPF2–KY295615, GenB–XPNPEP3–KY295742, GenB–ITS–KY294994); 1 ♂, Woogalong Homestead, Yalgoo (IBRA_MUR), 27°48′S, 116°34′E (WAM T27078); 1 ♂, same data (WAM T27079); 1 ♂, same data (WAM T27080); 1 ♂, Wubin (IBRA_AVW), 30°07′S, 116°38′E, 1 December 1936, H.K. Collins (WAM T2961); 1 ♂, Wubin, Rockdale (IBRA_AVW), 30°07′S, 116°38′E, 15 May 1965, R. Young (WAM T27053); 1 ♂, Wubin, Warrada Street (IBRA_AVW), 30°06′S, 116°38′E, A. Drew (WAM T143044); 1 ♂, Wyalkatchem (IBRA_AVW), 31°11′S, 117°23′E, on basketball court, in town, after storm, 1 January 1969, R. Hammond (WAM T27074); 1 ♂, same data (WAM T27075); 1 ♂, same locality data, 29 December 1970, M.J.C. Watt (WAM T27077); 1 ♂, same data except 17 December 1952, C.G. Jessup (WAM T143035); 1 ♂, same data except January 1981, K. Richards (WAM T143037); 1 ♂, Wyalkatchem, Box 58 (IBRA_AVW), 31°11′S, 117°23′E, 6 February 1960, Mrs. Remmont (WAM T27076); 1 ♂, Wyalkatchem Road, near Minnivale turnoff (IBRA_AVW), 31°11′S, 117°11′E, 22 January 1967, V. Blechendon (WAM T27073); 1 ♂, 20 km S. of Wyalkatchem (IBRA_AVW), 31°22′S, 117°23′E, 13–16 April 1998, K. Maitland (WAM T42165); 1 ♂, Yalgoo (YAL), 28°20′S, 116°40′E, 4 April 1923, S. Oliver (WAM T487); 1 ♂, Youanmi (IBRA_MUR), 28°37′S, 118°50′E, 24 December 1962, M.A. Edwards (WAM T27081).

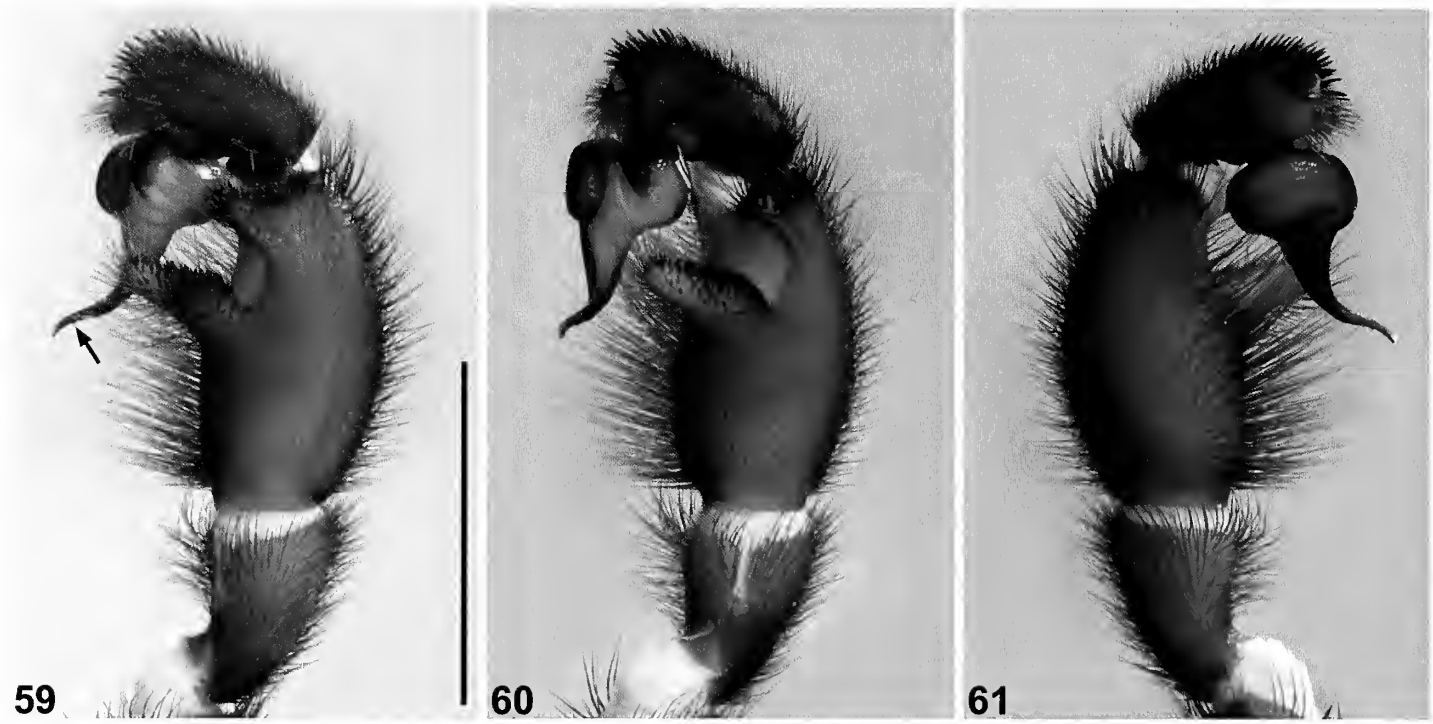
Diagnosis.—Males of *Gaius villosus* can be distinguished from those of *G. aurora* and *G. mainae* by the presence of prolateral clasping spurs on tibia I (Figs. 22–24; cf. Figs. 45, 133); from *G. austini* and *G. humphreysi* by the size and shape of the RTA, which is grossly enlarged (Fig. 25; cf. Figs. 59, 116); and from *G. cooperi*, *G. hueyi* and *G. tealei* by the size and shape of the distal retrolateral tibial apophysis (dRTA),



Figures 49–58.—*Gaius austini* sp. nov., male holotype (WAM T42166) from Coolgardie (Western Australia; COO), somatic morphology: 49–50, carapace and abdomen, dorsal view; 51, cephalothorax, lateral view; 52, eyes, dorsal view; 53, mouthparts, ventral view; 54–55, cephalothorax and abdomen, ventral view; 56, leg I, prolateral view; 57, leg I tibia, clasping spurs, prolateral view; 58, leg I tibia, proventral view. Scale bars = 5.0.

which is very large and 'ladle-shaped' (Fig. 25; cf. Figs. 81, 103, 156).

Description (male WAM T41700).—Total length 28.7. Carapace 12.2 long, 10.8 wide. Abdomen 10.7 long, 7.9 wide. Carapace (Fig. 15) broadly oval, dark chocolate-brown in color with mostly black ocular region; lateral margins densely setose; fovea slightly procurved. Eye group (Fig. 18) trapezoidal (anterior eye row strongly procurved), 0.6 x as long as wide, PLE–PLE/ALE–ALE ratio 1.6; ALE separated by 2.5 x their own diameter; AME separated by less than their own diameter; PME separated by 3.4 x their own diameter; PME and PLE separated by slightly more than diameter of PME, PME positioned slightly posterior to level of PLE. Maxillae with field of cuspules confined to inner corner; labium without cuspules. Abdomen (Fig. 16) oval, densely setose and dark brown in dorsal view, with paler beige-brown mottling;



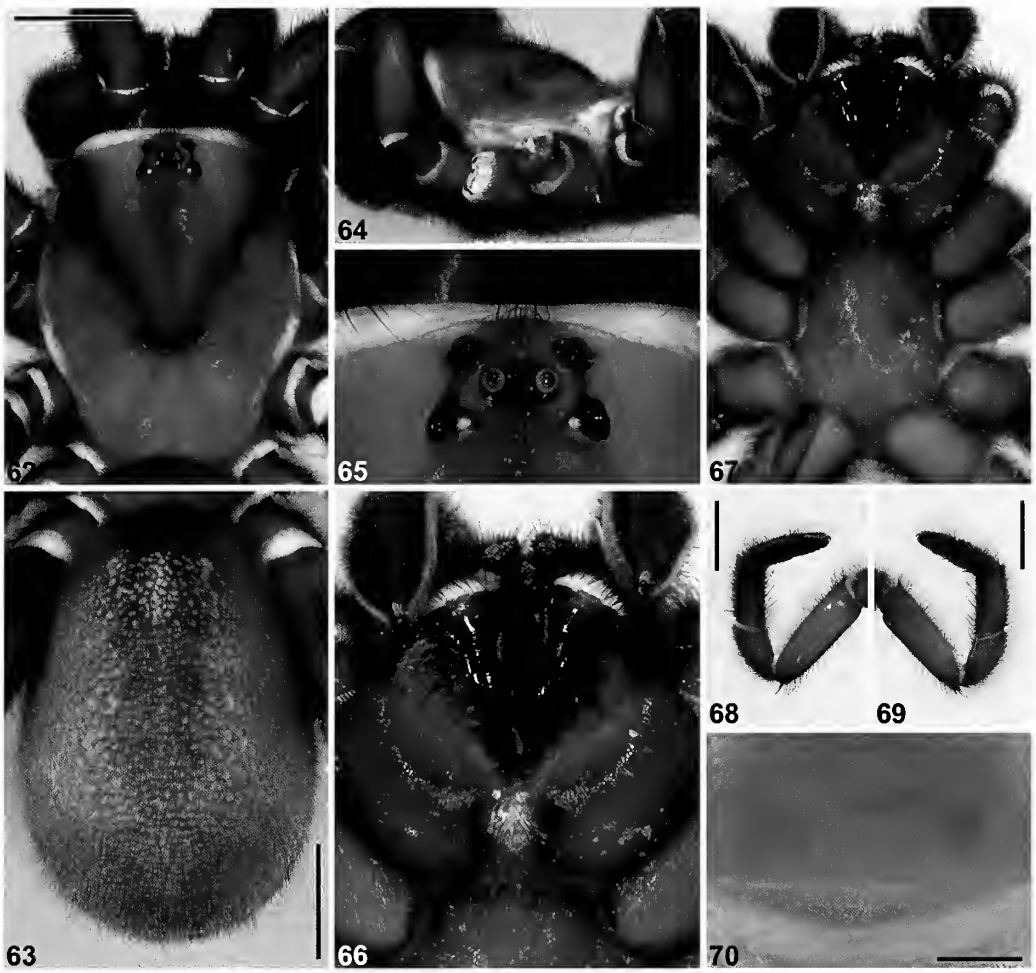
Figures 59–61.—*Gaius austini* sp. nov., male holotype (WAM T42166) from Coolgardie (Western Australia; COO), pedipalp: 59, retrolateral view (arrow to embolic apophysis); 60, retroventral view; 61, prolateral view. Scale bar = 5.0.

sclerotized sigilla absent. Legs (Figs. 22–24) variable shades of dark brown, with light scopulae on tarsi I–II; tibia I with row of 5 long retro-ventral macrosetae and distal pair of prolateral clasping spurs; metatarsus I with 3 pro-ventral and 9 retro-ventral macrosetae; tarsus I with 9 pro-ventral and 14 retro-ventral macrosetae. Leg I: femur 11.7; patella 5.4; tibia 8.2; metatarsus 7.2; tarsus 4.3; total 36.7. Leg I femur–tarsus/carapace length ratio 3.0. Pedipalpal tibia (Figs. 25–27) densely setose, 1.9 x longer than wide, with massive spinulate RTA and very large, 'ladle-shaped' distal retrolateral tibial apophysis (dRTA) also with sparse field of spinules. Cymbium (Figs. 25–27) setose, with field of weakly-developed spinules disto-dorsally. Embolus (Figs. 25–27) relatively broad at base, kinked medially and slightly twisted, with short, triangular embolic apophysis sub-distally.

Description (female WAM T132736).—Total length 38.7. Carapace 14.3 long, 12.4 wide. Abdomen 18.5 long, 16.2 wide. Carapace (Fig. 28) broadly oval, brown in color (dark chocolate-brown in life; Fig. 1) with mostly black ocular region; lateral margins densely setose; fovea procurved. Eye group (Fig. 31) trapezoidal (anterior eye row strongly procurved), 0.5 x as long as wide, PLE–PLE/ALE–ALE ratio 1.7; ALE separated by 2.3 x their own diameter; AME separated by approximately their own diameter; PME separated by 3.7 x their own diameter; PME and PLE separated by approximately 1.5 x diameter of PME, PME positioned slightly posterior to level of PLE. Maxillae with field of cuspules confined to inner corner; labium without cuspules. Abdomen (Fig. 29) broadly oval, beige-brown in dorsal view with darker cardiac marking (grey in life, with slate-grey cardiac region; Fig. 1); sclerotized sigilla absent. Legs (Figs. 34, 35) variable shades of dark brown, with thick scopulae on tarsi and metatarsi I–II; tibia I with cluster of 3 pro-distal macrosetae and row of 5 long retro-ventral macrosetae; metatarsus I with 4 ventral macrosetae; ventral tarsus I with distal cluster of 6 short macrosetae. Leg I: femur 9.9; patella 5.9; tibia 6.0; metatarsus 5.1; tarsus 3.5; total 30.5. Leg I femur–tarsus/carapace length ratio 2.1. Pedipalp dark brown, spinose on tibia, with thick tarsal scopula. Genitalia (Fig. 36) with pair of large, widely spaced, bud-shaped spermathecae on stalks (see also Main 1985, fig. 193).

Distribution and remarks.—*Gaius villosus* (formerly known, in part, by WAM identification codes 'MYG077' and 'MYG166') is a very large species with an extremely widespread distribution in arid Western Australia, extending from the central Wheatbelt, north through the southern Carnarvon, Murchison and southern Gascoyne bioregions, east to near Kalgoorlie (Fig. 11). Males (n = 101) generally wander in search of females in the warm (mostly summer) months of November–February (82% of collected specimens), usually after heavy thunderstorms, with a peak of activity in December and January (51% of collected specimens). If these storms do not occur, then males emerge with the first autumn/winter rains (Main 1987); no male specimens have ever been collected in September. For a detailed summary of the habits and natural history of this species, see Remarks under *Gaius* (above).

Conservation assessment.—This species has a known extent of occurrence of > 400,000 km^2, and is therefore not considered threatened under Criterion B. However, preliminary evidence suggests that population declines may have occurred in recent decades (Rix et al. 2017c), and further assessment under Criterion A is warranted in the future.



Figures 62–70.—*Gaius austini* sp. nov., female (WAM T96059) from Marvel Loch (Western Australia; COO): 62–63, carapace and abdomen, dorsal view; 64, cephalothorax, lateral view; 65, eyes, dorsal view; 66, mouthparts, ventral view; 67, cephalothorax, ventral view; 68, leg I, prolateral view; 69, leg I, retrolateral view; 70, spermatheeae, dorsal view. Scale bars = 5.0 (62–63, 68–69), 1.0 (70).

Gaius aurora sp. nov.
http://zoobank.org/?lsid=urn:lsid:zoobank.org:act:A5F52EB9-8D05-4EDB-9F1D-AFBD454D488B
(Figs. 11, 14, 37–48)

Type material.—*Holotype male*. AUSTRALIA: *Western Australia*: Bungalbin Hill, Helena-Aurora Range, site BHR5 (IBRA_COO), 30°17′S, 119°43′E, hand collected, 1 December 1980, Goldfields Survey (WAM T16076).

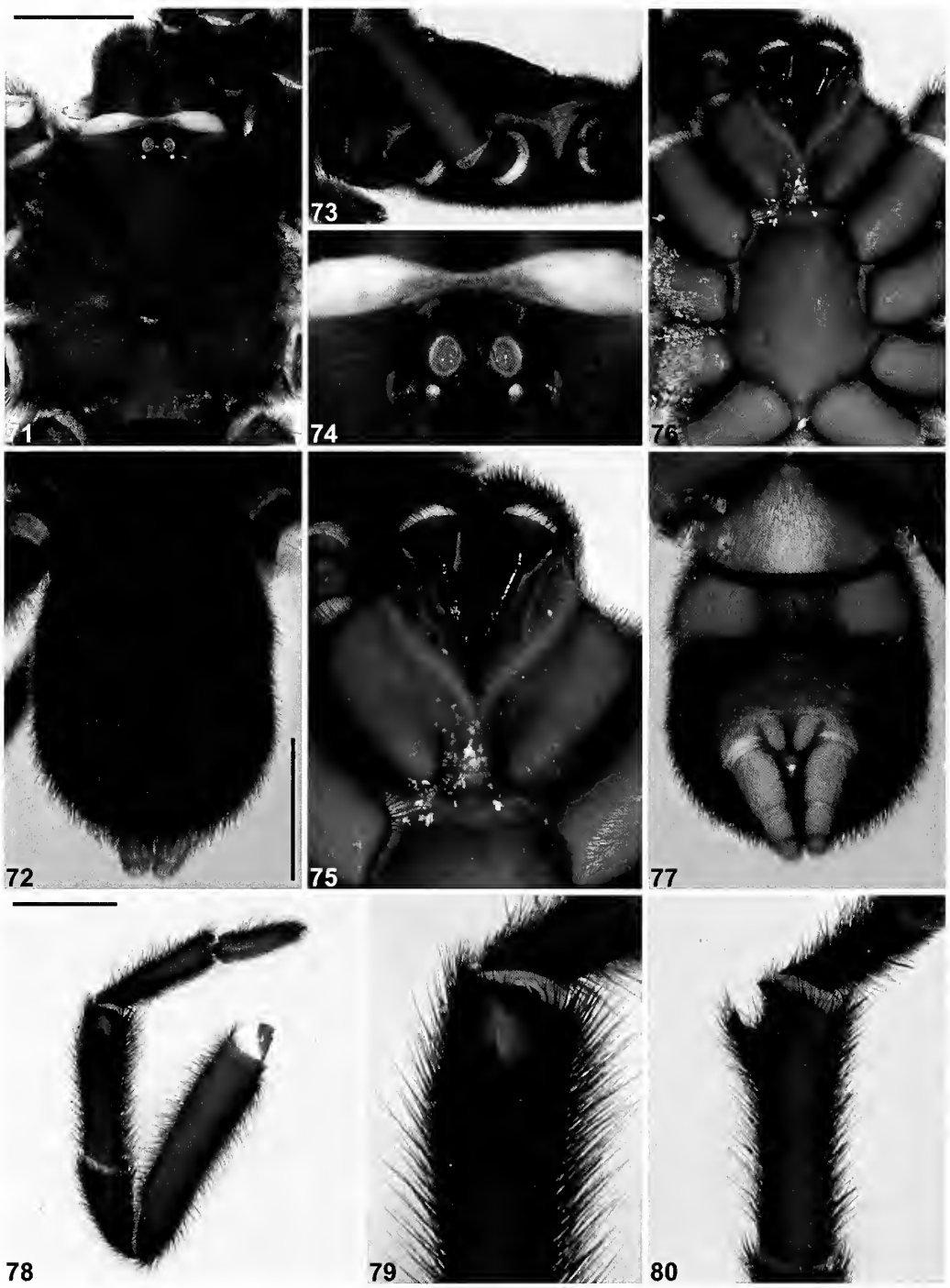
Paratypes. AUSTRALIA: *Western Australia*: 1 ♂, same data as holotype except 1 April 1980 (WAM T16067); 1 ♂, same data (WAM T16068); 1 ♂, same data (WAM T16069); 1 ♂, same data (WAM T16070); 1 ♂, same data except site BHR3 (WAM T16065); 1 ♂, same data (WAM T16066).

Other material examined.—AUSTRALIA: *Western Australia*: 1 ♂, Bungalbin Hill, Helena-Aurora Range (IBRA_COO), 30°16′11″S, 119°46′30″E], wet pitfall trap, November 1995, M.A. Cowan (WAM T34265); 1 ♂, same data (WAM T34266); 1 ♂, Bungalbin Hill, 25 km N. of sandplain (IBRA_COO), 20 February 1989, C. Dickman (WAM T143059).

Etymology.—The specific epithet is a noun in apposition, in reference to the type locality of this species.

Diagnosis.—Males of *Gaius aurora* can be distinguished from those of all other congeners except *G. mainae* by the absence of prolateral clasping spurs on tibia I (Figs. 44–45; cf. Figs. 23, 57, 79, 101, 114, 154); and from *G. mainae* by the size and shape of the RTA, which is much smaller (Fig. 46; cf. Fig. 134).

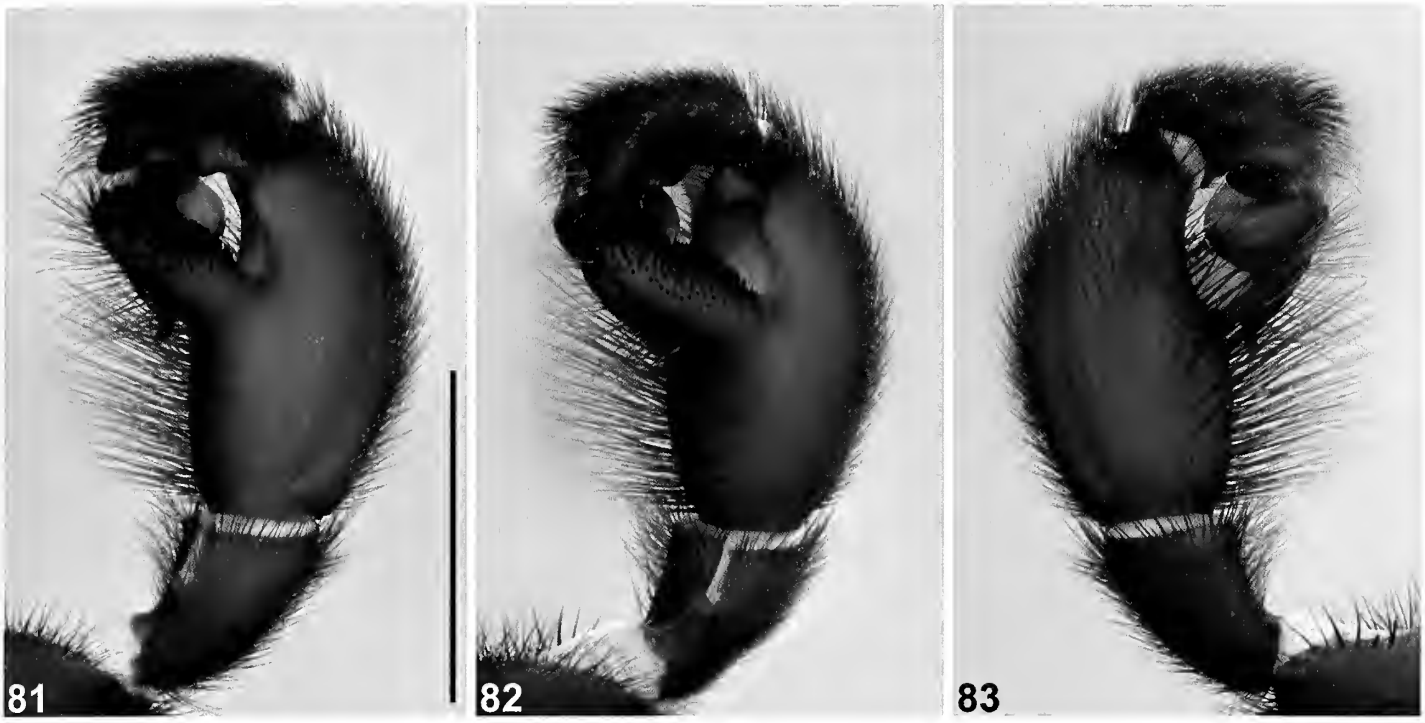
Description (male holotype).—Total length 29.2. Carapace 11.5 long, 10.0 wide. Abdomen 12.3 long, 7.6 wide. Carapace (Fig. 37) oval, dark chocolate-brown in color with mostly black ocular region; lateral margins densely setose; fovea slightly procurved. Eye group (Fig. 40) trapezoidal (anterior eye row strongly procurved), 0.6 x as long as wide, PLE–PLE/ALE–ALE ratio 1.5; ALE separated by 1.9 x their own diameter; AME separated by less than their own diameter; left PME missing; right PME and PLE separated by approximately 2.0 x diameter of right PME, right PME positioned in line with level of PLE. Maxillae with field of cuspules confined to inner corner; labium without cuspules. Abdomen (Fig. 38) oval, densely setose and dark grey-brown in dorsal view, with paler beige-brown mottling; sclerotized sigilla absent. Legs (Figs. 44, 45) variable shades of dark brown, with light scopulae on tarsi I–II; tibia I with pro-distal comb of macrosetae and row of 7 long retro-ventral macrosetae;



Figures 71–80.—*Gaius cooperi* sp. nov., male holotype (WAM T96604) from Forrestania, 84.3 km E. of Hyden (Western Australia; COO), somatic morphology: 71–72, carapace and abdomen, dorsal view; 73, cephalothorax, lateral view; 74, eyes, dorsal view; 75, mouthparts, ventral view; 76–77, cephalothorax and abdomen, ventral view; 78, leg I, prolateral view; 79, leg I tibia, clasping spurs, prolateral view; 80, leg I tibia, proventral view. Scale bars = 5.0.

metatarsus I with 2 pro-ventral and 5 retro-ventral macrosetae. Leg I: femur 10.6; patella 5.1; tibia 7.1; metatarsus 6.7; tarsus 4.1; total 33.6. Leg I femur–tarsus/carapace length ratio 2.9. Pedipalpal tibia (Figs. 46–48) densely setose, 2.2 x longer than wide, with relatively small spinulate RTA and large, 'finger-like' distal retrolateral tibial apophysis (dRTA) also with sparse field of spinules. Cymbium (Figs. 46–48) setose, with field of weakly-developed spinules disto-dorsally. Embolus (Figs. 46–48) slightly twisted and gently tapered, with very short, triangular embolic apophysis sub-distally.

Distribution and remarks.—*Gaius aurora* is a large species with a restricted distribution in the Helena-Aurora Range (northern Coolgardie bioregion) of Western Australia, with most specimens known from the vicinity of Bungalbin Hill (approximately 115 km NNE. of Southern Cross) (Fig. 11). Nothing is known of the biology of this species, other than



Figures 81–83.—*Gaius cooperi* sp. nov., male holotype (WAM T96604) from Forrestania, 84.3 km E. of Hyden (Western Australia: COO), pedipalp: 81, retrolateral view; 82, retroventral view; 83, prolateral view. Scale bar = 5.0.

that the known male specimens (n = 10) were collected wandering in search of females in November–December (30%), February (10%) and April (60%). Females are unknown.

Conservation assessment.—This rare species has a known extent of occurrence (EOO) of < 200 km^2, and an area of occupancy (AOO) within that range of < 10 km^2. While both of these figures are undoubtedly underestimates due to fairly limited sampling in the greater Helena-Aurora Range, good sampling from surrounding areas suggests that the EOO is likely to be < 10,000 km^2, and almost certainly << 20,000 km^2. Similarly, based on those surveys that have occurred in the region, the likely AOO is calculated at < 500 km^2. Given this geographic range, the occurrence of the species at < 10 known sites, and the continuing decline in the area, extent and/or quality of habitat due to current or proposed mining exploration in surrounding areas, this species is considered Vulnerable (B1ab[iii] + B2ab[iii]).

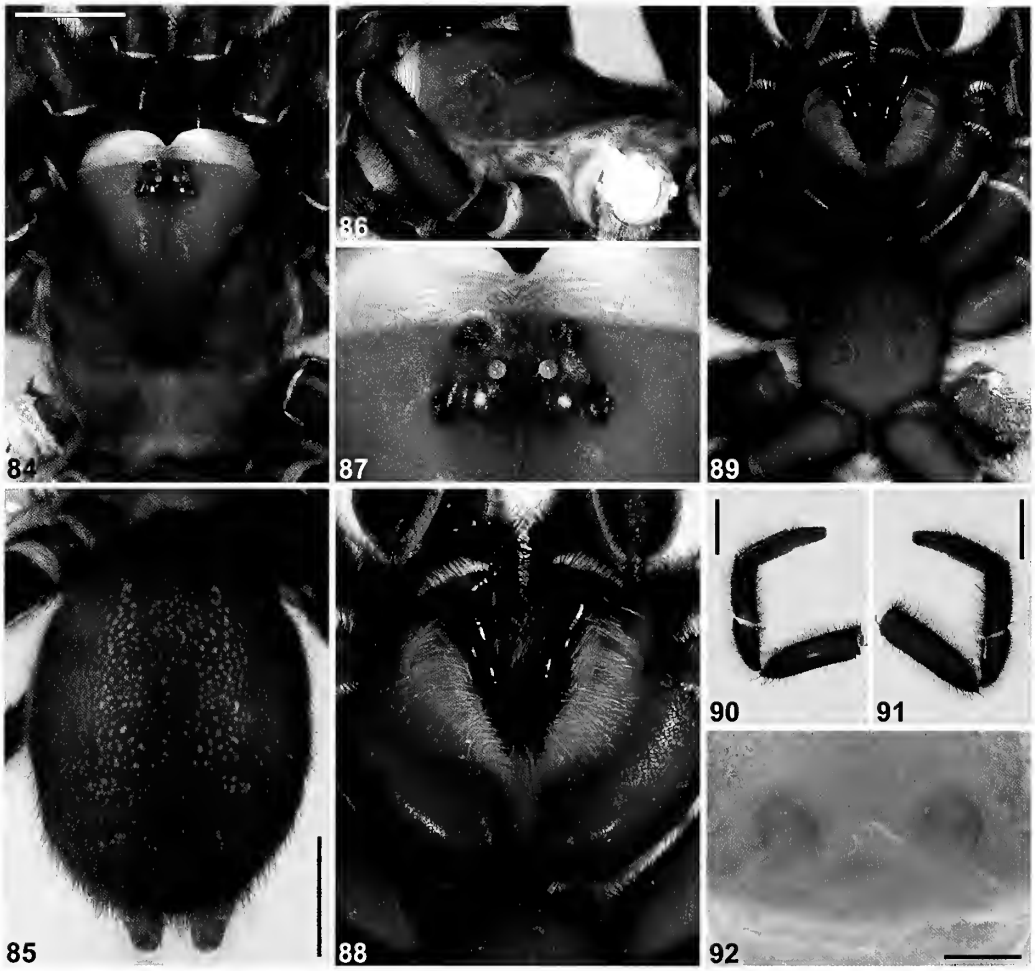
Gaius austini sp. nov.
http://zoobank.org/?lsid=urn:lsid:zoobank.org:act:AACDBBEE-53C5-44FD-8E9A-64DEED6D7123
(Figs. 5, 11, 14, 49–70)

Gaius sp. 'Kalgoorlie' Rix et al., 2017d: 619, figs. 240, 241, 243, 247, 249.

Type material.—*Holotype male*. AUSTRALIA: *Western Australia*: Coolgardie, 32 Hunt Street (IBRA_COO), 30°57′S, 121°09′E, 16 December 1996, R. & J. Kippin (WAM T42166).

Paratypes. AUSTRALIA: *Western Australia*: 1 ♂, Coolgardie (IBRA_COO), 30°57′S, 121°10′E, 11 January 1987, M. Charlton (WAM T27007); 1 ♂, same data except hand collected, 29 December 1993, R. Edwards (WAM T29775); 1 ♂, same locality data, 13 January 2000, I. Keally (WAM T41550); 1 ♂, same locality data except 71 Forrest Street, 28 January 2001, W. Moore (WAM T44217); 1 ♂, same locality data except Forrest Street, 21 February 1997, M. Billing (WAM T44178); 1 ♂, same locality data except 8 King Street, 30°57′S, 121°09′E, 18 December 1996, S. Moore (WAM T41697).

Other material examined.—AUSTRALIA: *Western Australia*: 1 ♂, Boulder (IBRA_COO), 30°47′S, 121°29′E, hand collected, 18 March 1993, R. Ugle (WAM T27981); 1 ♂, same locality data except 193 Western Road, 22 December 1995, J. Hutchinson (WAM T44177); 1 ♂, Chalice Goldmine, ca. 50 km N. of Norseman (IBRA_COO), 31°45′S, 121°47′E, 1 February 1999, M. Ryan (WAM T38858); 1 ♂, Credo Station (IBRA_COO), 30°28′S, 120°50′E, December 1985, E. Halford (WAM T27009); 1 ♀, same locality data except 30°01′43″S, 120°39′00″E, dug from burrow, 3 September 2011, M.S. Harvey (WAM T116013^{DNA_Voucher_NCB_027}; GenB–COI–MG652494; GenB–MRPL45–MG652548, GenB–RPF2–MG652568, GenB–XPNPEP3–MG652588, GenB–ITS–MG652530); 1 ♀, Woodland south of Helena Aurora Range, ca. 80 km NE. of Bullfinch (IBRA_COO), 30°23′49.55″S, 119°47′51.32″E, 17 November 2015, M.K. Curran, D. Harms (WAM T140952^{DNA_Voucher_NCB_026}; GenB–COI–MG652495); 1 ♂, Kalgoorlie (IBRA_COO), 30°45′S, 121°28′E, 30 December 1991, P. Tregatt (WAM T26991); 1 ♂, same data (WAM T26992); 1 ♂, same data except found at night in defensive posture, 30 November 1987, R. Black (WAM T27020); 1 ♂, same locality data, January 1986, D. Pearson (WAM T27021); 1 ♂, same data except 25 February 1994, Dept. of CALM (WAM T29939); 1 ♂, same data except 19 January 1994 (WAM T30021); 1 ♂, same data except 19 January 1995, Museum of The Goldfields (WAM T31789); 1



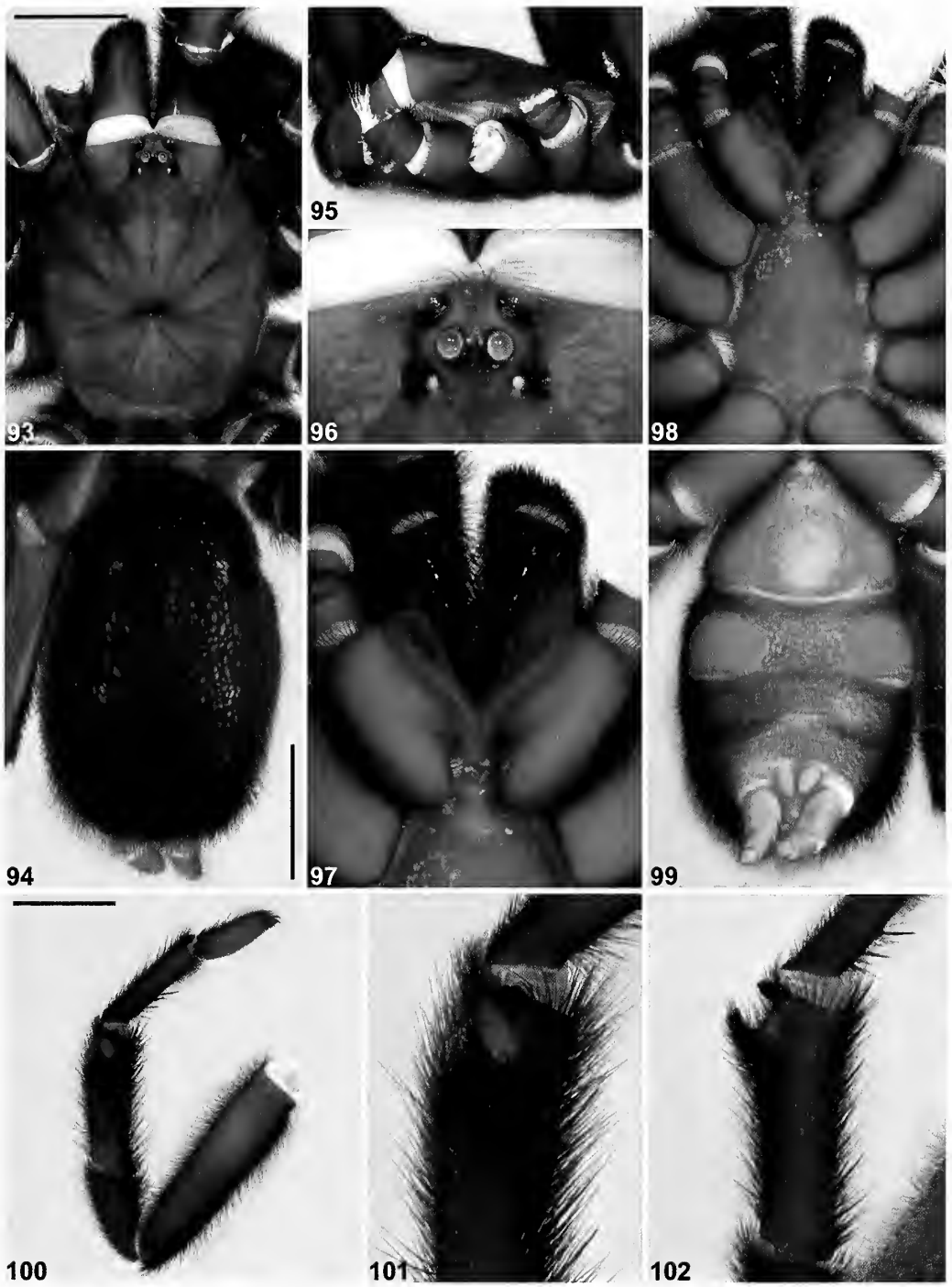
Figures 84–92.—*Gaius cooperi* sp. nov., female (WAM T144017) from Westralia Conservation Park (Western Australia; JAF): 84–85, carapace and abdomen, dorsal view; 86, cephalothorax, lateral view; 87, eyes, dorsal view; 88, mouthparts, ventral view; 89, cephalothorax, ventral view; 90, leg I, prolateral view; 91, leg I, retrolateral view; 92, spermatheeae, dorsal view. Scale bars = 5.0 (84–85, 90–91), 1.0 (92).

♂, same locality data except Dugan Street, 7 December 1996, T. Moller (WAM T41699); 1 ♂, same locality data except Hannan Subdivision, inside house, 5 December 1994, D. Brams (WAM T31788); 1 ♂, same locality data, 6 January 1989, K. Parker (WAM T38857); 1 ♂, same locality data except Hare Street, 22 December 1995, E. Robertson (WAM T44171); 1 ♂, same locality data except rubbish tip, 5 December 1994, R. Utterson (WAM T31787); 1 ♂, 10 km E. of Kalgoorlie (IBRA_COO), 30°43′55″S, 121°31′55″E, dry pitfall trap, salmon gum low woodland, dwarf scrub on loam, 29 November 2014, G.P. Harewood (WAM T136255); 1 ♂, same data except 30°44′40″S, 121°34′01″E, blackbutt low woodland over open scrub on loam, 30 January 2014 (WAM T136256); 1 ♂, same data except 30°46′06″S, 121°33′44″E, sheoak forest tree mallee over low scrub on loam (WAM T136257); 1 ♀, ca. 50 km ESE. of Kalgoorlie (IBRA_COO), 30°53′58″S, 121°56′15″E, 8–12 November 2010, S.A. Thompson (WAM T109002^{DNA_Voucher_288}; GenB–CYB–MG652512, GenB–MRPL45–MG652549, GenB–RPF2–MG652570, GenB–XPNPEP3–MG652590, GenB–ITS–MG652532); 1 ♀, Marvel Loch, St Barbara Operation, Cornishman area, site 18 (IBRA_COO), 31°16′16″S, 119°22′12″E, dug from burrow, 1 August 2008, P. Cullen, P. Langlands (WAM T96059^{DNA_Voucher_285}; GenB–MRPL45–MG652550, GenB–RPF2–MG652569, GenB–XPNPEP3–MG652589, GenB–ITS–MG652531); 1 ♂, Mount Burges Station Homestead (IBRA_COO), 30°50′S, 121°06′E, eucalypt woodland on red clay soil, 15 December 1987, D. Egerton-Warburton (WAM T27041).

Etymology.—This species is named in honor of Professor Andy Austin (of the University of Adelaide), in recognition of his contributions to idiopid systematics, and his role in developing and overseeing the Australian Research Council (ARC) idiopid project.

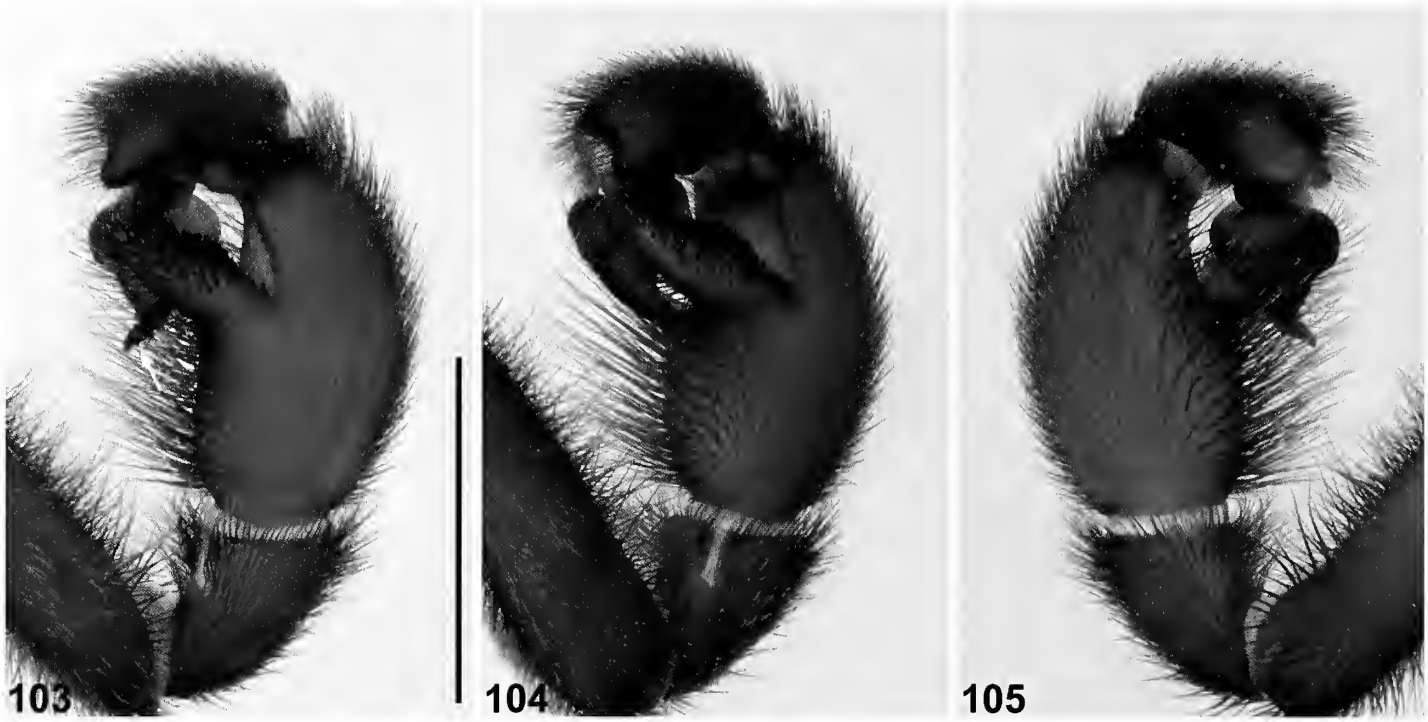
Diagnosis.—Males of *Gaius austini* can be distinguished from those of *G. aurora* and *G. maiae* by the presence of prolateral clasping spurs on tibia I (Figs. 56–58; cf. Figs. 45, 133); from *G. cooperi*, *G. hueyi*, *G. tealei* and *G. villosus* by the size and shape of the RTA, which is not grossly enlarged (Fig. 59; cf. Figs. 25, 81, 103, 156); and from *G. humphreysi* by the inter-distance of the ALE, which are separated by approximately twice their own diameter (Fig. 52; cf. Fig. 109), combined with the morphology of the embolic apophysis, which is positioned sub-distally (Figs. 59–61).

Description (male holotype).—Total length 33.4. Carapace 12.6 long, 10.0 wide. Abdomen 14.7 long, 9.9 wide. Carapace



Figures 93–102.—*Gaius hueyi* sp. nov., male holotype (WAM T32171) from Munglinup (Western Australia; ESP). somatic morphology: 93 94, carapace and abdomen, dorsal view; 95, cephalothorax, lateral view; 96, eyes, dorsal view; 97, mouthparts, ventral view; 98 99, cephalothorax and abdomen, ventral view; 100, leg I, prolateral view; 101, leg I tibia, clasping spurs, prolateral view; 102, leg I tibia, proventral view. Scale bars = 5.0.

(Fig. 49) roughly hexagonal, dark tan-brown in color with mostly black ocular region; lateral margins densely setose; fovea procurved. Eye group (Fig. 52) trapezoidal (anterior eye row strongly procurved), 0.6 x as long as wide, PLE–PLE/ALE–ALE ratio 1.4; ALE separated by 2.0 x their own diameter; AME separated by less than their own diameter; PME separated by 4.6 x their own diameter; PME and PLE separated by approximately 1.5 x diameter of PME, PME positioned in line with level of PLE. Maxillae with field of cuspules confined to inner corner; labium without cuspules. Abdomen (Fig. 50) oval, densely setose and dark grey-brown in dorsal view, with paler beige-brown mottling; sclerotized sigilla absent. Legs (Figs. 56–58) variable shades of dark brown, with light scopulae on tarsi I–II; tibia I with distal pair of prolateral clasping spurs; metatarsus I with 2 pro-ventral and 4 retro-ventral macrosetae. Leg I: femur 10.6; patella 5.5;



Figures 103–105.—*Gaius hueyi* sp. nov., male holotype (WAM T32171) from Munglinup (Western Australia; ESP), pedipalp: 103, retrolateral view (arrow to embolic apophysis); 104, retroventral view; 105, prolateral view. Scale bar = 5.0.

tibia 7.5; metatarsus 6.8; tarsus 4.1; total 34.5. Leg I femur–tarsus/carapace length ratio 2.7. Pedipalpal tibia (Figs. 59–61) densely setose, 1.9 x longer than wide, with proximally-widened, spinulate RTA and broad, subrectangular distal retrolateral tibial apophysis (dRTA) also with field of spinules. Cymbium (Figs. 59–61) setose, with field of spinules disto-dorsally. Embolus (Figs. 59–61) curved, gently tapered, with short, triangular embolic apophysis sub-distally.

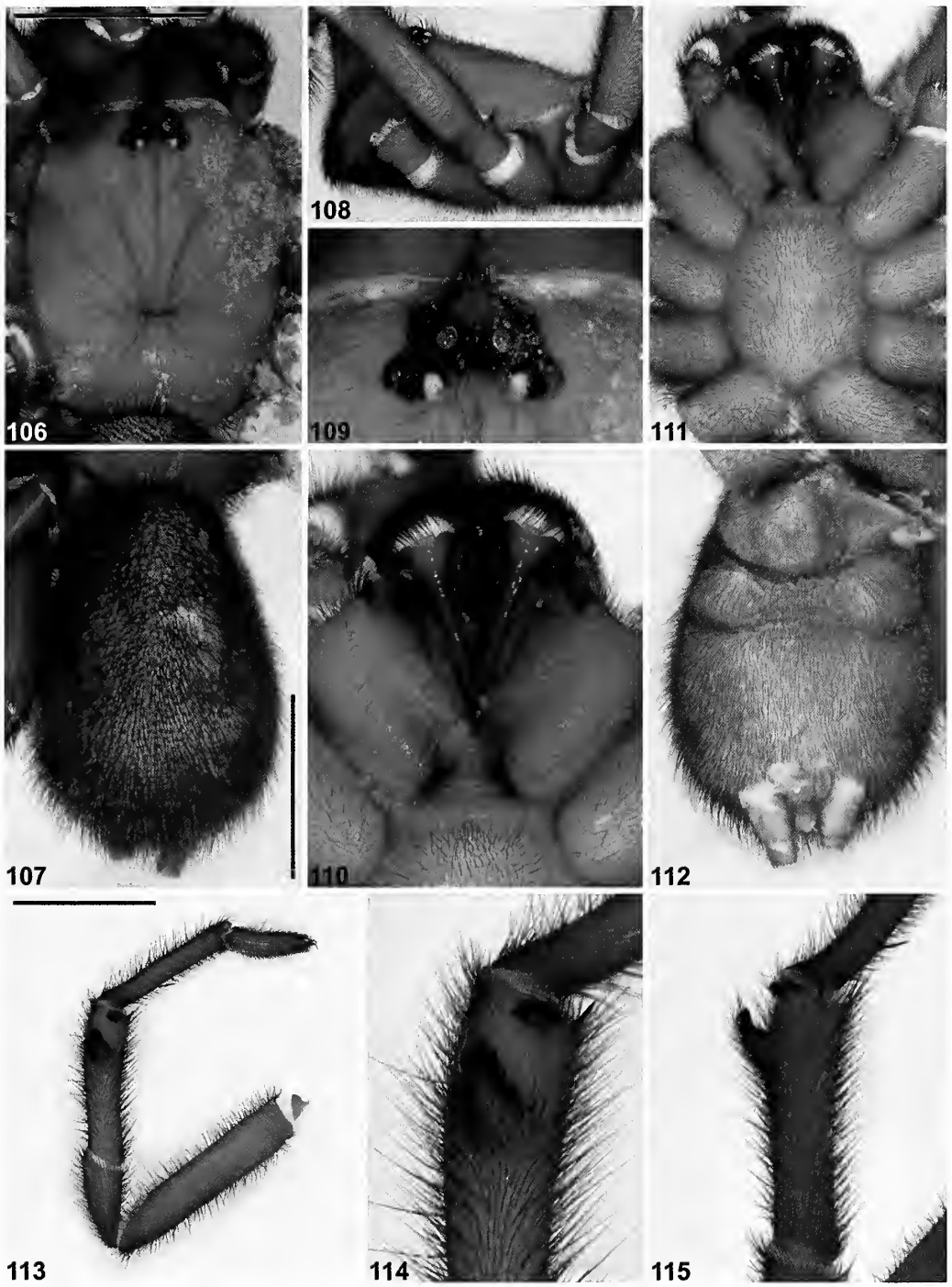
Description (female WAM T96059).—Total length 36.9. Carapace 13.7 long, 10.7 wide. Abdomen 19.1 long, 12.9 wide. Carapace (Fig. 62) roughly hexagonal, dark tan-brown in color with mostly black ocular region; lateral margins densely setose; fovea procurved. Eye group (Fig. 65) trapezoidal (anterior eye row strongly procurved), 0.5 x as long as wide, PLE–PLE/ALE–ALE ratio 1.3; ALE separated by 2.0 x their own diameter; AME separated by approximately their own diameter; PME separated by 5.8 x their own diameter; PME and PLE separated by approximately diameter of PME, PME positioned in line with level of PLE. Maxillae with field of cuspules confined to inner corner; labium without cuspules. Abdomen (Fig. 63) oval, grey-brown in dorsal view, with paler beige-brown mottling and darker cardiac marking; sclerotized sigilla absent. Legs (Figs. 68, 69) variable shades of dark brown, with thick scopulae on tarsi and metatarsi I–II; tibia I with cluster of 2 pro-distal macrosetae and row of 5 long retro-ventral macrosetae; metatarsus I with 6 ventral macrosetae; ventral tarsus I with distal cluster of approximately 7 short macrosetae. Leg I: femur 8.1; patella 5.0; tibia 4.7; metatarsus 3.8; tarsus 2.9; total 24.4. Leg I femur–tarsus/carapace length ratio 1.8. Pedipalp dark brown, spinose on tibia, with thick tarsal scopula. Genitalia (Fig. 70) with pair widely spaced, bud-shaped spermathecae.

Distribution and remarks.—*Gaius austini* is a large species with a widespread distribution in the Coolgardie bioregion of southern inland Western Australia, from Southern Cross and the Helena-Aurora Range east to at least Kalgoorlie and Lake Cowan (Fig. 11). Based on collection records, this species is not uncommon around Coolgardie and Kalgoorlie, where its range overlaps the south-eastern extent of the range of *G. villosus*. In the west of its range, *G. austini* also overlaps with *G. mainae* and possibly also *G. aurora*. *Gaius austini* is closely related to *G. humphreysi* from the northern Murchison (Fig. 13), with which it shares a relatively small RTA and small spermathecae (compared to most other species of *Gaius*). Males (n = 23) wander in search of females in the warm (mostly summer) months of November–March (100% of collected specimens), presumably after heavy rain, with a peak of activity in December (43% of collected specimens).

Conservation assessment.—This species has a known extent of occurrence of > 30,000 km^2, and is therefore not considered threatened under Criterion B. However, preliminary evidence suggests that population declines may have occurred among arid zone Idiopidae in recent decades (Rix et al. 2017c), and further assessment under Criterion A is warranted in the future.

Gaius cooperi sp. nov.
http://zoobank.org/?lsid=urn:lsid:zoobank.org:act:C24017B1-127B-4D9A-AAA8-957D459062F3
(Figs. 4, 9, 11, 14, 71–92)
Gaius sp. 'Collie' Rix et al., 2017d: 619, figs. 245, 250.

Type material.—*Holotype male*. AUSTRALIA: *Western Australia*: Forrestania, 84.3 km E. of Hyden (IBRA_COO), 32°21′50″S, 119°45′30″E, dug from burrow, 18–25 January 2008, P. Runham (WAM T96604^{DNA_Voucher_220}; GenB–

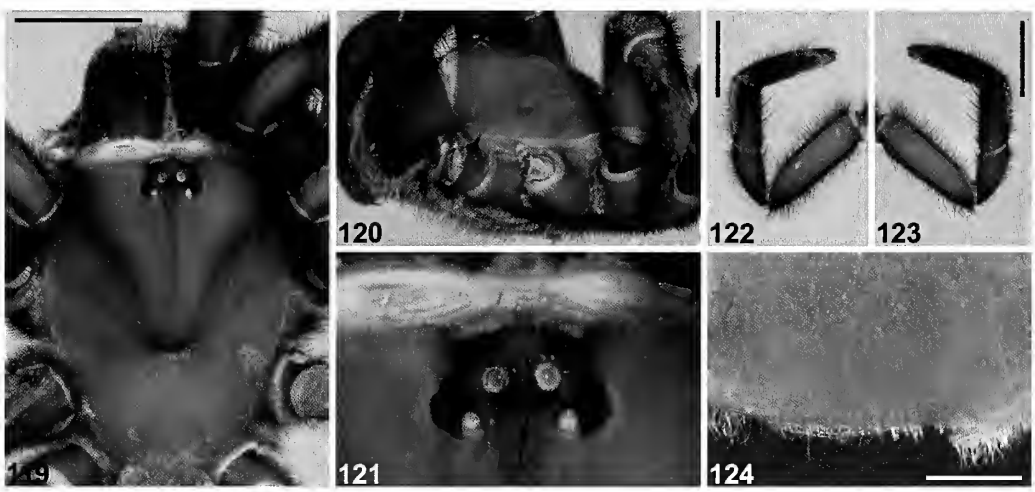


Figures 106–115.—*Gaius humphreysi* sp. nov., male holotype (WAM T96563) from Mount Keith Mine (Western Australia; MUR), somatic morphology: 106–107, carapace and abdomen, dorsal view; 108, cephalothorax, lateral view; 109, eyes, dorsal view; 110, mouthparts, ventral view; 111–112, cephalothorax and abdomen, ventral view; 113, leg 1, prolateral view; 114, leg 1 tibia, clasping spurs, prolateral view; 115, leg 1 tibia, proventral view. Scale bars = 5.0.

CYB–MG652509, GenB–MRPL45–MG652535, GenB–RPF2–MG652554, GenB–XPNPEP3–MG652574, GenB–ITS–MG652515).

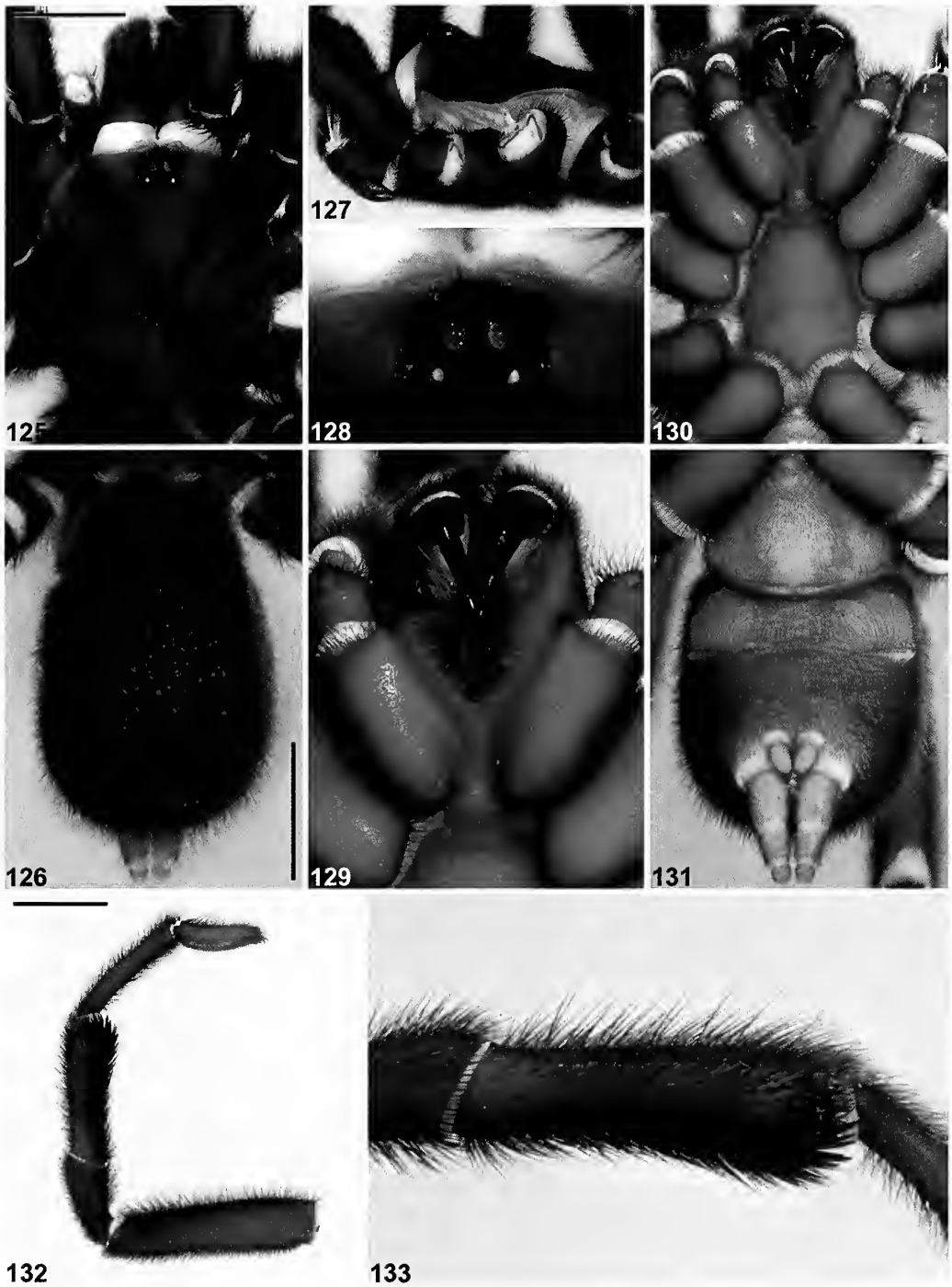
Other material examined.—AUSTRALIA: *Western Australia*: 1 ♂, Allanson (IBRA_JAF), 33°20′S, 116°06′E, February 1992, W. Pool (WAM T27085); 1 ♂, 80 km E. to SE. of Bruce Rock (IBRA_AVW), 31°52′S, 119°00′E, 21 January 1982, M. Gray (WAM T26999); 1 ♂, Camel Lake Nature Reserve, east, site ST 7 (IBRA_ESP), 34°15′59″S, 117°58′44″E, wet pitfall traps, 15 October 1999–1 November 2000, P. Van Heurck et al., CALM Survey (WAM T143054); 1 ♂, same data except south, site ST 4, 34°17′34″S, 117°58′51″E, 15 October 1999–25 November 2000, B. Durrant, CALM Survey (WAM T143055); 1 ♂, same data (WAM T143056); 1 ♂, Collie (IBRA_JAF), 33°21′S, 116°09′E, 16 January 1971, Br. Kelly (WAM T27005); 1 ♂, same locality data, on lawn, March

Figures 116–118.—*Gaius humphreysi* sp. nov., male holotype (WAM T96563) from Mount Keith Mine (Western Australia; MUR), pedipalp; 116, retrolateral view (arrow to embolic apophysis); 117, retroventral view; 118, prolateral view. Scale bar = 5.0.



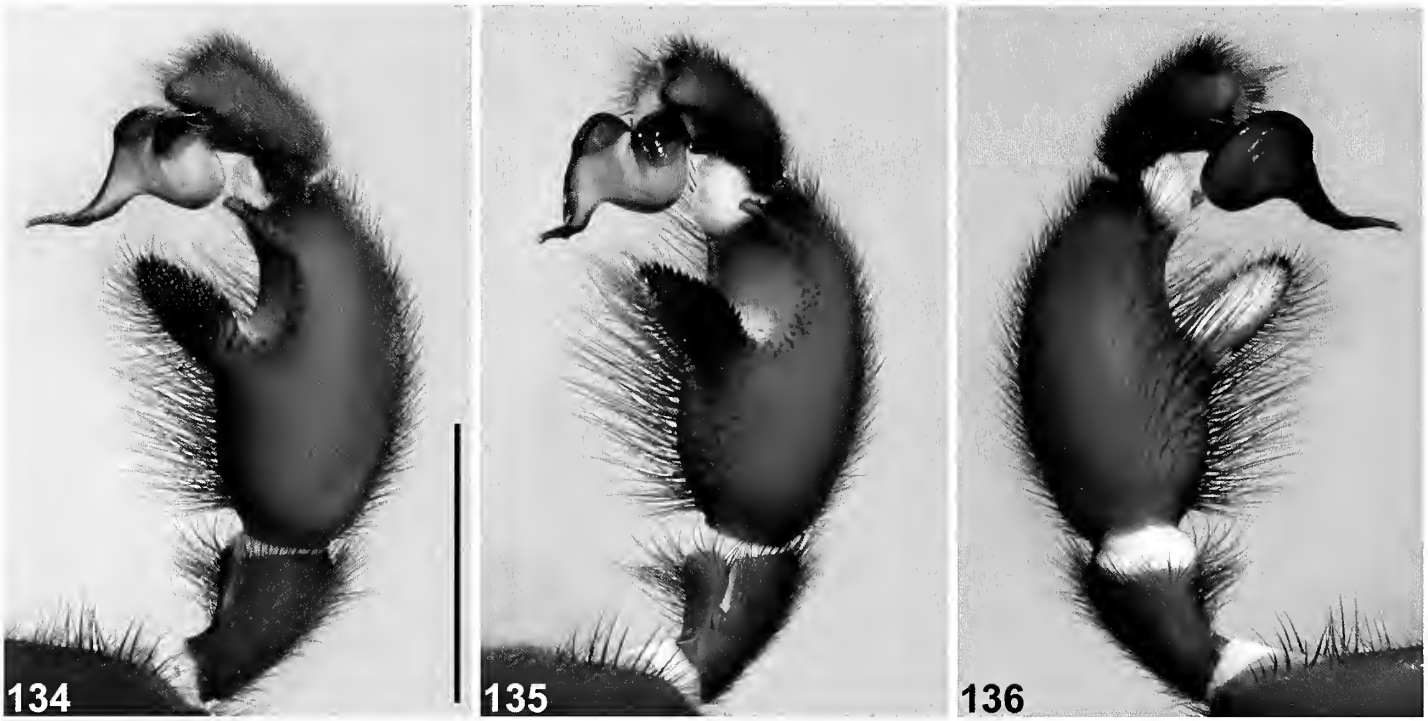
Figures 119–124.—*Gaius humphreysi* sp. nov., female (WAM T98757) from De La Poer Nature Reserve (Western Australia; MUR); 119, carapace, dorsal view; 120, cephalothorax, lateral view; 121, eyes, dorsal view; 122, leg I, prolateral view; 123, leg I, retrolateral view; 124, spermatheeae, dorsal view. Note that the abdomen of this specimen is severely damaged and the ventral cephalothorax is obscured by dried hemolymph. Scale bars = 5.0 (119, 122–123), 1.0 (124).

1987, D. Peters (WAM T27006); 1 ♂, same locality data except Park Street, April 2006, C. Letica (WAM T143045); 1 ♂, Cordinup, Green Range, 6328 (IBRA_ESP), 34°40′S, 118°25′E, 23 March 1982, B. Long (WAM T27015); 1 ♂, Gulson Lake, site HY 8 (IBRA_MAL), 32°47′11″S, 119°22′07″E, wet pitfall traps, 30 October 1997–20 May 1998, E. Ladhams, CALM Survey (WAM T143053); 1 ♂, Hyden (IBRA_MAL), 32°27′S, 118°52′E, 22 February 1979, P.A. Mulcahy (WAM T27017); 1 ♂, same data except 9 March 1988, N. Fotheringham (WAM T27018); 1 ♂, Lake King (IBRA_MAL), 33°05′08″S, 119°40′42″E, 24 January 1990, C. Kimbler (WAM T20546); 1 ♂, 10 km E. of Lake Varley (IBRA_MAL), 32°42′S, 119°29′E, 5 February 1976, G. Barron (WAM T27084); 1 ♂, McDermid Rock, site MRR2 (IBRA_COO), 32°03′S, 120°42′E, pitfall trap, *Callitris campestris*/heath, 11–17 February 1981, W.F. Humphreys et al. (WAM T16177); 1 ♂, same data except site MRR3, shrubland (WAM T16178); 1 ♂, same data (WAM T16199); 1 ♂, same data (WAM T16200); 1 ♂, Newdegate (IBRA_MAL), 33°06′S, 119°01′E, 7 April 1989, C. Thompson (WAM T26990); 1 ♂, same data except 5 March 1974, E.T. Richardson (WAM T27046); 1 ♂, Orana, Albany Highway



Figures 125–133.—*Gaius mainae* sp. nov., male holotype (WAM T40696) from Grass Patch (Western Australia; MAL), somatic morphology: 125–126, carapace and abdomen, dorsal view; 127, cephalothorax, lateral view; 128, eyes, dorsal view; 129, mouthparts, ventral view; 130–131, cephalothorax and abdomen, ventral view; 132, leg I, prolateral view; 133, leg I tibia, prolateral view. Scale bars = 5.0.

near Anson Road (IBRA_JAF), 35°00′S, 117°51′E, 10 January 2006, A.D. Brown (WAM T76089); 1 ♂, Pinjalup Road, E. of Tenterden, site ST 2 (IBRA_JAF), 34°21′44″S, 117°34′07″E, wet pitfall traps, 15 October 1999–30 November 2000, B. Durrant, CALM Survey (WAM T143046); 1 ♂, same data except site ST 3, 34°21′38″S, 117°33′43″E, 15 October 1999–1 November 2000, P. Van Heurek et al., CALM Survey (WAM T143047); 1 ♂, same data (WAM T143048); 1 ♂, same data (WAM T143049); 1 ♂, Pyramid Lake, east, ca. 14 km NW. of Grass Patch, site GP 4 (IBRA_MAL), 33°09′31″S, 121°00′03″E, wet pitfall traps, 15 October 1999–26 November 2000, B. Durrant, CALM Survey (WAM T143052); 1 ♂, Tambellup (IBRA_AVW), 34°03′S, 117°39′E, hand collected, June 1953, R. Mawsen (WAM T3725); 1 ♂, same locality data, 16–17 February 1989, J. Cavanagh (WAM T27082); 1 ♂, Tenterden (IBRA_JAF), 34°22′S, 117°33′E, hand collected, 25



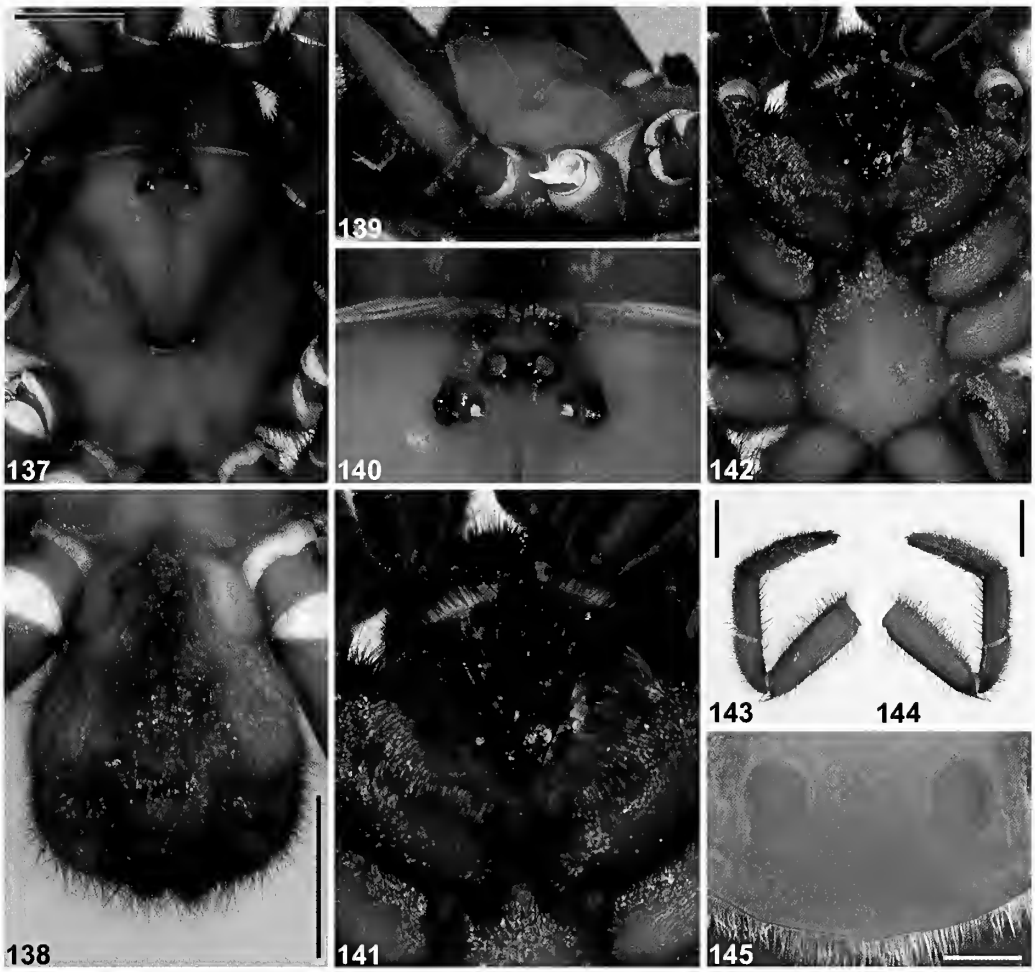
Figures 134–136.—*Gaius mainae* sp. nov., male holotype (WAM T40696) from Grass Patch (Western Australia: MAL), pedipalp: 134, retrolateral view; 135, retroventral view; 136, prolateral view. Scale bar = 5.0.

March 1993, D.M. Sandstrom (WAM T27983); 1 ♂, same locality data, 9 March 2001, J. Cavanagh (WAM T44157[DNA_Voucher_215]; GenB–CYB–MG652510, GenB–MRPL45–MG652536, GenB–RPF2–MG652556, GenB–XPNPEP3–MG652585, GenB–ITS–MG652517); 1 ♀, Voyager Quarry, Mundaring Shire, ca. 3 km NE. of The Lakes (IBRA_JAF), 31°51′59.7″S, 116°21′03.2″E, dug from burrow, 11 July 2007, P. Runham, T. Schmidt (WAM T143511[DNA_Voucher_NCB_023]; GenB–COI–MG652496, GenB–RPF2–MG652553, GenB–XPNPEP3–MG652573, GenB–ITS–MG652514); 1 juvenile, same data (WAM T143521); 1 ♀, same data except 31°51′59.5″S, 116°21′03.1″E (WAM T143512[DNA_Voucher_NCB_028]; GenB–MRPL45–MG652533, GenB–RPF2–MG652552, GenB–XPNPEP3–MG652572, GenB–ITS–MG652513); 1 ♀, same data except 31°51′59.3″S, 116°21′03.2″E (WAM T143516); 1 juvenile, same data except 31°51′59.1″S, 116°21′03.1″E (WAM T143513); 1 juvenile, same data (WAM T143514); 1 juvenile, same data except 31°51′59.7″S, 116°21′03.3″E (WAM T143515); 1 juvenile, same data except 31°51′59.3″S, 116°21′03.1″E (WAM T143517); 1 juvenile, same data except 31°51′59.2″S, 116°21′03.2″E (WAM T143519); 1 juvenile, same data except 31°51′59.8″S, 116°21′03.2″E (WAM T143520); 1 ♀, Westralia Conservation Park, ca. 5 km W. of Collie (IBRA_JAF), 33°20′56″S, 116°06′55″E, from burrow, ca. 40 cm deep, 10 August 2017, J.A. Huey, M.S. Harvey (WAM T144017[DNA_Voucher_NCB_024]; GenB–MRPL45–MG652534, GenB–RPF2–MG652555, GenB–XPNPEP3–MG652582, GenB–ITS–MG652516).

Etymology.—This species is named in honor of Professor Steve Cooper (of the South Australian Museum), in recognition of his contributions to idiopid systematics, and his role in developing the Australian Research Council (ARC) idiopid project.

Diagnosis.—Males of *Gaius cooperi* can be distinguished from those of *G. aurora* and *G. mainae* by the presence of prolateral clasping spurs on tibia 1 (Figs. 78–80; cf. Figs. 45, 133); from *G. austini* and *G. humphreysi* by the size and shape of the RTA, which is grossly enlarged (Fig. 81; cf. Figs. 59, 116); and from *G. hueyi*, *G. tealei* and *G. villosus* by the size and shape of the distal retrolateral tibial apophysis (dRTA), which is hooked, with a constricted proximal 'stem' and variably-shaped, distally widened 'knob' (Fig. 81; cf. Figs. 25, 103, 156).

Description (male holotype).—Total length 35.1. Carapace 12.7 long, 10.4 wide. Abdomen 12.9 long, 8.4 wide. Carapace (Fig. 71) broadly oval, dark chocolate-brown in color with mostly black ocular region; lateral margins densely setose; fovea slightly procurved. Eye group (Fig. 74) trapezoidal (anterior eye row strongly procurved), 0.6 x as long as wide, PLE–PLE/ALE–ALE ratio 1.5; ALE separated by 1.8 x their own diameter; AME separated by less than their own diameter; PME separated by 3.8 x their own diameter; PME and PLE separated by approximately 2.0 x diameter of PME, PME positioned in line with level of PLE. Maxillae with field of cuspules confined to inner corner; labium without cuspules. Abdomen (Fig. 72) oval, densely setose and dark grey-brown in dorsal view, with paler beige-brown mottling and two pairs of faint posterior chevrons; sclerotized sigilla absent. Legs (Figs. 78–80) variable shades of dark brown, with light scopulae on tarsi I–II and distal third of metatarsi I–II; tibia 1 with row of 5 long retro-ventral macrosetae and distal pair of prolateral clasping spurs; metatarsus I with 2 pro-ventral and 3 retro-ventral macrosetae. Leg 1: femur 11.2; patella 5.7; tibia

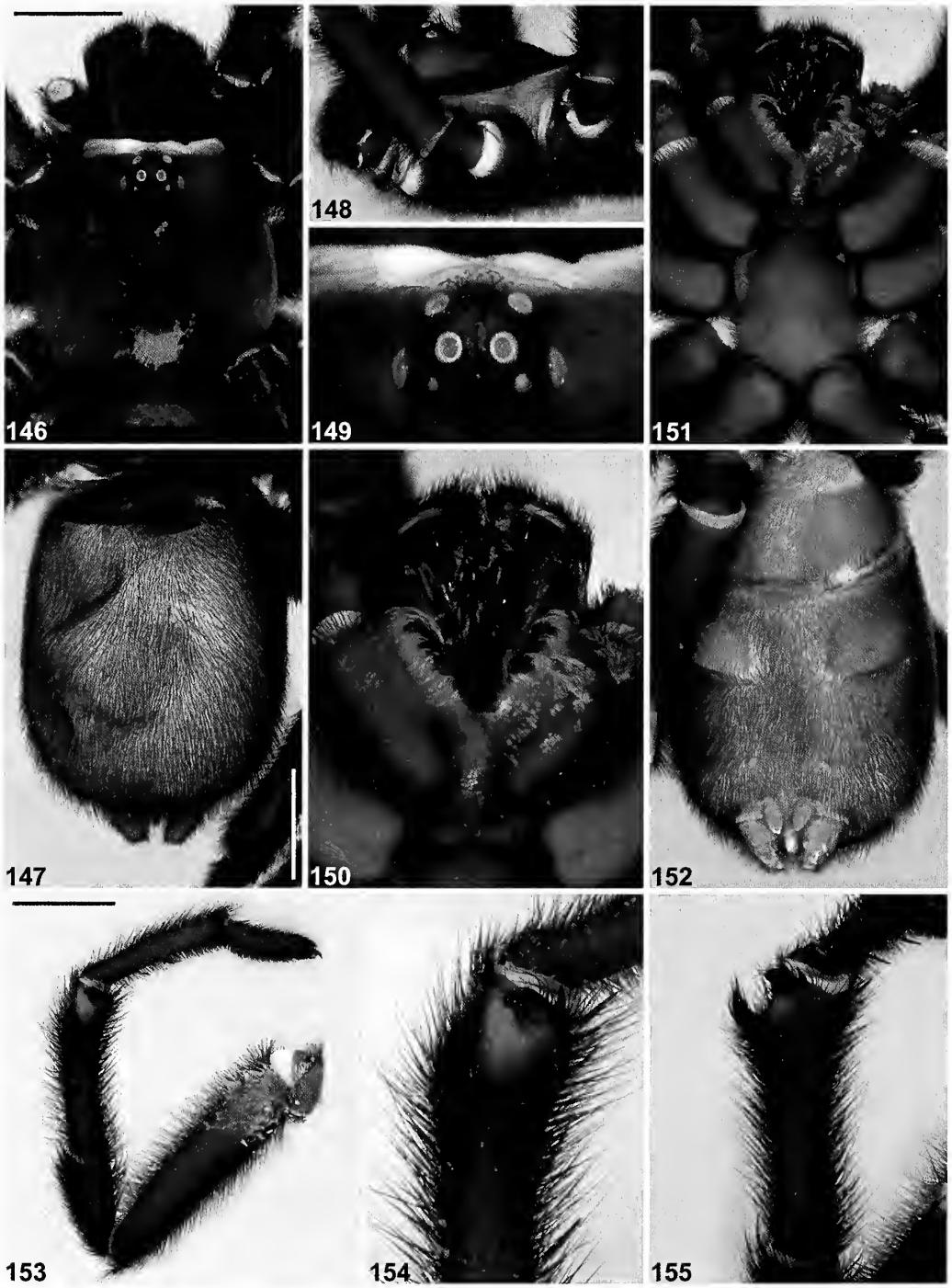


Figures 137–145.—*Gaius maimae* sp. nov., female paratype (WAM T41380) from Grass Patch (Western Australia: MAL): 137–138, carapace and abdomen, dorsal view; 139, cephalothorax, lateral view; 140, eyes, dorsal view; 141, mouthparts, ventral view; 142, cephalothorax, ventral view; 143, right leg I, prolateral view (flipped horizontal); 144, right leg I, retrolateral view (flipped horizontal); 145, spermatheeae, dorsal view. Scale bars = 5.0 (137–138, 143–144), 1.0 (145).

7.5; metatarsus 6.6; tarsus 4.1; total 35.1. Leg I femur–tarsus/carapace length ratio 2.8. Pedipalpal tibia (Figs. 81–83) densely setose, 2.0 x longer than wide, with massive spinulate RTA and large, hooked distal retrolateral tibial apophysis (dRTA) with proximal 'stem', distally widened 'knob' and sparse field of spinules. Cymbium (Figs. 81–83) setose, with field of weakly-developed spinules disto-dorsally. Embolus (Figs. 81–83) relatively broad at base and twisted, with short, triangular embolic apophysis sub-distally.

Description (female WAM T144017).—Total length 36.8. Carapace 13.7 long, 11.2 wide. Abdomen 16.5 long, 11.7 wide. Carapace (Fig. 84) oval, dark brown in color (dark brown-black in life; Fig. 4) with mostly black ocular region; posterior pars cephalica and lateral margins densely setose; fovea procurved. Eye group (Fig. 87) trapezoidal (anterior eye row strongly procurved), 0.6 x as long as wide, PLE–PLE/ALE–ALE ratio 1.4; ALE separated by 2.8 x their own diameter; AME separated by slightly more than their own diameter; PME separated by 4.4 x their own diameter; PME and PLE separated by approximately 2.0 x diameter of PME, PME positioned in line with level of PLE. Maxillae with field of cuspules confined to inner corner; labium without cuspules. Abdomen (Fig. 85) oval, dark brown in dorsal view (dark brown in life; Fig. 4), with paler beige-brown mottling and two pairs of faint posterior chevrons; sclerotized sigilla absent. Legs (Figs. 90, 91) variable shades of dark brown, with thick scopulae on tarsi and metatarsi I–II; tibia I with cluster of 7 pro-distal and 2 retro-ventral macrosetae; metatarsus I with 1 pro-ventral and 3 retro-ventral macrosetae; ventral tarsus 1 with distal cluster of 4 short macrosetae. Leg I: femur 9.3; patella 5.3; tibia 5.4; metatarsus 5.2; tarsus 3.2; total 28.5. Leg I femur–tarsus/carapace length ratio 2.1. Pedipalp dark brown, spinose on tibia, with thick tarsal scopula. Genitalia (Fig. 92) with pair of large, widely spaced, bud-shaped spermathecae.

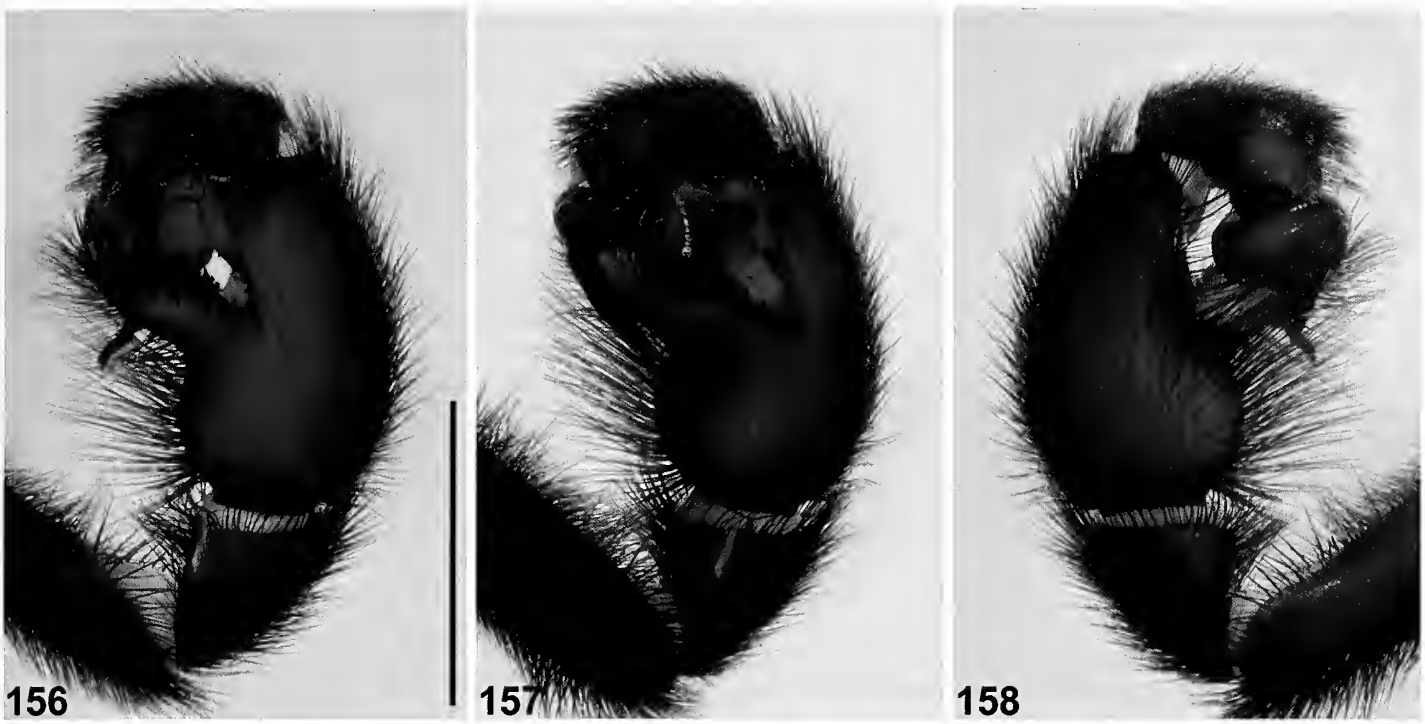
Distribution and remarks.—*Gaius cooperi* (formerly known by WAM identification code 'MYG063') is a large species with a widespread distribution in the Mallee and surrounding bioregions of southern Western Australia, from Collie, Tenterden and Albany in the west, north to Voyager Quarry and Holleton, and east to McDermid Rock and Pyramid Lake (Fig. 11). However, based on collection records, this species appears to occur in three disjunct zones: around Collie and Voyager Quarry in the Jarrah Forest; around Albany,



Figures 146–155.—*Gaius tealei* sp. nov., male holotype (WAM T117550) from Lorna Glen Station (Western Australia; MUR), somatic morphology: 146–147, carapace and abdomen, dorsal view; 148, cephalothorax, lateral view; 149, eyes, dorsal view; 150, mouthparts, ventral view; 151–152, cephalothorax and abdomen, ventral view; 153, leg I, prolateral view; 154, leg I tibia, clasping spurs, prolateral view; 155, leg I tibia, proventral view. Scale bars = 5.0.

Tenterden, Tambellup and Cordinup in the Great Southern region; and in the zone demarcated by Newdegate, Holleton, McDermid Rock and Pyramid Lake (Fig. 11). Males ($n = 21$) wander in search of females in the warm (mostly summer) months of January–April (95% of collected specimens), presumably after heavy rain, with a peak of activity in February and March (62% of collected specimens).

Conservation assessment.—This species has a known extent of occurrence of approximately 100,000 km^2, and is therefore not considered threatened under Criterion B. However, preliminary evidence suggests that population declines may have occurred among arid zone Idiopidae in recent decades (Rix et al. 2017c), and further assessment under Criterion A is warranted in the future. Indeed, the Voyager Quarry



Figures 156–158.—*Gaius tealei* sp. nov., male holotype (WAM T117550) from Lorna Glen Station (Western Australia: MUR), right pedipalp (flipped horizontal for comparison): 156, retrolateral view; 157, retroventral view; 158, prolateral view. Scale bar = 5.0.

population, near The Lakes (east of Perth), is possibly now extinct due to expanded mining activity at the only known location.

Gaius hueyi sp. nov.
http://zoobank.org/?lsid=urn:lsid:zoobank.org:act:C3C3714E-5306-4B49-8ED9-9CC0B7B2A1B7
(Figs. 11, 14, 93–105)

Type material.—*Holotype male.* AUSTRALIA: *Western Australia*: Munglinup (IBRA_ESP), 33°42′S, 120°51′E, hand collected, 12 January 1995, R. Davis (WAM T32171).

Paratypes. AUSTRALIA: *Western Australia*: 1 ♂, Munglinup Beach (IBRA_ESP), 33°53′S, 120°48′E, hand collected under *Melaleuca*, 25 March 1995, M. Jeffery (WAM T32555); 1 ♂, 3 km N. of mouth of Munglinup River (W. of Esperance) (IBRA_ESP), 33°40′S, 120°51′E, at night, moving over dead grass/granite, 8 March 1988, P.J. Fuller (WAM T27045).

Other material examined.—AUSTRALIA: *Western Australia*: 1 ♂, Esperance (IBRA_ESP), 33°51′S, 121°53′E, 5 March 1974, P.K. Arlidge (WAM T29850); 1 ♂, Esperance area (IBRA_ESP), 33°51′S, 121°53′E, 5 February 1999, S. Perks (WAM T41698); 1 ♂, Fields Road, S. of Griffiths Road, site GP 12 (IBRA_MAL), 33°28′31″S, 121°14′10″E, wet pitfall traps, 15 October 1999–1 November 2000, P. Van Heurck et al., CALM Survey (WAM T143051); 2 ♂, Jerdacuttup, location 829 (IBRA_ESP), 33°42′S, 120°28′E, 23 February 1987, G. Boothey (WAM T143057); 1 ♂, 11 km W. of Point Dempster (IBRA_ESP), 33°38′S, 123°44′E, 11 March 1984, R.A. How (WAM T27051); 1 ♂, Ravensthorpe, Springvale Road (IBRA_ESP), 33°35′S, 120°03′E, hand collected, 22 March 1993, G. Boothey (WAM T27982).

Etymology.—This species is named in honor of Dr. Joel Huey (of the Western Australian Museum), in recognition of his contributions to mygalomorph systematics, and for assisting with the sequencing component of this study.

Diagnosis.—Males of *Gaius hueyi* can be distinguished from those of *G. aurora* and *G. mainae* by the presence of prolateral clasping spurs on tibia 1 (Figs. 100–102; cf. Figs. 45, 133); from *G. austini* and *G. humphreysi* by the size and shape of the RTA, which is grossly enlarged (Fig. 103; cf. Figs. 59, 116); and from *G. cooperi*, *G. tealei* and *G. villosus* by the size and shape of the distal retrolateral tibial apophysis (dRTA), which is not hooked, and forms a simple, rounded, disto-ventrally directed process (Fig. 103; cf. Figs. 25, 81, 156).

Description (male holotype).—Total length 31.6. Carapace 12.7 long, 10.3 wide. Abdomen 13.9 long, 8.9 wide. Carapace (Fig. 93) oval, dark tan-brown in color with mostly black ocular region; lateral margins densely setose; fovea slightly procurved. Eye group (Fig. 96) trapezoidal (anterior eye row strongly procurved), 0.7 x as long as wide, PLE–PLE/ALE–ALE ratio 1.5; ALE separated by 1.5 x their own diameter; AME separated by less than their own diameter; PME separated by 5.0 x their own diameter; PME and PLE separated by approximately 1.5 x diameter of PME, PME positioned in line with level of PLE. Maxillae with field of cuspules confined to inner corner; labium without cuspules. Abdomen (Fig. 94) oval, densely setose and dark grey-brown in dorsal view, with paler beige-brown mottling and two pairs of faint posterior chevrons; sclerotized sigilla absent. Legs (Figs. 100–102) variable shades of dark brown, with light scopulae on tarsi I–II and distal third of metatarsi I–II; tibia I with row of 6 long retro-ventral macrosetae and distal pair of prolateral clasping spurs; metatarsus I with 6 pro-ventral and 5 retro-ventral macrosetae. Leg 1: femur 10.8; patella 5.3; tibia

7.1; metatarsus 6.2; tarsus 3.9; total 33.3. Leg I femur–tarsus/carapace length ratio 2.6. Pedipalpal tibia (Figs. 103–105) densely setose, 1.9 x longer than wide, with massive spinulate RTA and large, rounded, disto-ventrally directed distal retrolateral tibial apophysis (dRTA) also with sparse field of proximal spinules. Cymbium (Figs. 103–105) setose, with field of weakly-developed spinules disto-dorsally. Embolus (Figs. 103–105) relatively broad at base and twisted, with short, triangular embolic apophysis sub-distally.

Distribution and remarks.—*Gaius hueyi* is a large species with a restricted distribution in the Esperance Plains bioregion of southern Western Australia, from Ravensthorpe east to Point Dempster (Fig. 11). Males ($n = 8$) generally wander in search of females in the warm (mostly summer) months of January–March (100% of collected specimens), presumably after heavy rain, with a possible peak of activity in March (63% of collected specimens). Females are unknown.

Conservation assessment.—This rare species has a known extent of occurrence (EOO) of approximately 14,000 km^2, and an estimated area of occupancy (AOO) within that range of < 2,000 km^2. Given this geographic range, the occurrence of the species at < 10 severely fragmented sites, and the continuing decline in the area, extent and/or quality of habitat due largely to fungal dieback disease (caused by *Phytophthora*), this species is considered Vulnerable (B1ab[iii] + B2ab[iii]).

Gaius humphreysi sp. nov.
http://zoobank.org/?lsid=urn:lsid:zoobank.org:act:82765493-426E-4809-840B-D655D4113802
(Figs. 11, 14, 106–124)

Type material.—*Holotype male.* AUSTRALIA: *Western Australia*: Mount Keith Mine, 82.5 km SE. of Wiluna (IBRA_MUR), 27°16′32″S, 120°33′48″E, 12 November 2006, R. Teale, Z. Hamilton (WAM T96563^{DNA_Voucher_222}; GenB–COI–MG652497).

Other material examined.—AUSTRALIA: *Western Australia*: 1 ♀, De La Poer Nature Reserve (IBRA_MUR), 27°24′18.97″S, 122°43′33.85″E, 10 September 2009, B. Durrant, M. Hoskins (WAM T98757^{DNA_Voucher_289}; GenB–COI–MG652498, GenB–CYB–MG652511, GenB–MRPL45–MG652551, GenB–RPF2–MG652571, GenB–XPNPEP3–MG652591, GenB–ITS–MG652529).

Other material examined (tentatively assigned).—AUSTRALIA: *Western Australia*: 1 ♂, Caiguna (IBRA_COO), 32°16′S, 125°29′E, 4 March 1971, E.A. Odell (WAM T26984).

Etymology.—This species is named in honor of Garth Humphreys (of Biota Environmental Sciences), in recognition of his support of the Australian Research Council (ARC) idiopid project since its commencement in 2012.

Diagnosis.—Males of *Gaius humphreysi* can be distinguished from those of *G. aurora* and *G. mainae* by the presence of prolateral clasping spurs on tibia I (Figs. 113–115; cf. Figs. 45, 133); from *G. cooperi*, *G. hueyi*, *G. tealei* and *G. villosus* by the size and shape of the RTA, which is not grossly enlarged (Fig. 116; cf. Figs. 25, 81, 103, 156); and from *G. austini* by the inter-distance of the ALE, which are separated by approximately their own diameter (Fig. 109; cf. Fig. 52), combined with the morphology of the embolic apophysis, which is positioned distally (Figs. 116, 118). See remarks below for information regarding an aberrant male specimen from Caiguna (WAM T26984), which may not be conspecific with this species.

Description (male holotype).—Total length 20.1. Carapace 8.4 long, 6.6 wide. Abdomen 9.7 long, 6.7 wide. Carapace (Fig. 106) oval, tan-brown in color with mostly black ocular region; lateral margins densely setose; fovea slightly procurved. Eye group (Fig. 109) trapezoidal (anterior eye row strongly procurved), 0.6 x as long as wide, PLE–PLE/ALE–ALE ratio 1.5; ALE separated by 1.3 x their own diameter; AME separated by less than their own diameter; PME separated by 2.8 x their own diameter; PME and PLE separated by approximately diameter of PME, PME positioned slightly posterior to level of PLE. Maxillae and labium without cuspules. Abdomen (Fig. 107) oval, densely setose and dark grey in dorsal view, with paler beige-grey mottling; sclerotized sigilla absent. Legs (Figs. 113–115) variable shades of tan-brown, with light scopulae on tarsi I–II; tibia I with distal pair of prolateral clasping spurs; metatarsus I with 2 pro-ventral and 2 retro-ventral macrosetae. Leg I: femur 7.5; patella 3.7; tibia 5.2; metatarsus 5.4; tarsus 2.7; total 24.5. Leg I femur–tarsus/carapace length ratio 2.9. Pedipalpal tibia (Figs. 116–118) densely setose, 1.8 x longer than wide, with porrect, spinulate RTA and broad, subquadrate distal retrolateral tibial apophysis (dRTA) also with field of spinules. Cymbium (Figs. 116–118) setose, with field of spinules disto-dorsally. Embolus (Figs. 116–118) curved, gently tapered, with distal, triangular embolic apophysis forming distinctly expanded tip.

Description (female WAM T98757).—Total length approximately 32.0 (abdomen severely damaged). Carapace 12.2 long, 9.4 wide. Abdomen approximately 14.7 long (severely damaged). Carapace (Fig. 119) roughly hexagonal, dark tan-brown in color with mostly black ocular region; lateral margins densely setose; fovea procurved. Eye group (Fig. 121) trapezoidal (anterior eye row strongly procurved), 0.6 x as long as wide, PLE–PLE/ALE–ALE ratio 1.5; ALE separated by approximately their own diameter; AME separated by approximately their own diameter; PME separated by 3.6 x their own diameter; PME and PLE separated by approximately diameter of PME, PME positioned slightly posterior to level of PLE. Maxillae and labium obscured by dried hemolymph. Abdomen severely damaged. Legs (Figs. 122, 123) variable shades of dark brown, with thick scopulae on tarsi and metatarsi I–II; tibia I with cluster of 2 pro-distal and 2 ventral macrosetae, and row of 5 long retro-ventral macrosetae; metatarsus I with 6 ventral macrosetae; ventral tarsus I with distal cluster of short macrosetae, obscured by dried hemolymph. Leg I: femur 7.7; patella 4.7; tibia 4.4; metatarsus 4.1; tarsus 2.8; total 23.7. Leg I femur–tarsus/carapace length ratio 1.9. Pedipalp dark brown, spinose on tibia, with thick tarsal scopula. Genitalia (Fig. 124) with pair widely spaced, bud-shaped spermathecae on short stalks.

Distribution and remarks.—*Gaius humphreysi* (formerly known by WAM identification code 'MYG061') is a rare, medium-large species with a poorly known distribution in the north-eastern Murchison bioregion, from Mount Keith east to at least the De La Poer Nature Reserve (Fig. 11). A single isolated specimen from Caiguna in the far eastern Coolgardie bioregion (Fig. 11), which has a very similar pedipalp morphology to the holotype (see Supplementary File 1, online at http://dx.doi.org/10.1636/JoA-S-17-079.s1), is also tenta-

tively included here, but is larger, with more widely spaced ALEs; additional specimens and/or molecular data are required to confirm the identification of this population. *Gaius humphreysi* is closely related to *G. austini* from the Coolgardie bioregion (Fig. 13), with which it shares a relatively small RTA and small spermathecae (compared to most other species of *Gaius*). Nothing is known of the biology of this species, other than that the holotype male specimen was collected in November, and the tentatively assigned Caiguna specimen was collected in March.

Conservation assessment.—Due to the known occurrence of this species at only two (or possibly three; see above) widely separated localities, we consider it data deficient for the purposes of conservation assessment.

Gaius mainae sp. nov.
http://zoobank.org/?lsid=urn:lsid:zoobank.org:act:E6C6090E-8217-4388-B00B-47D09A76471F
(Figs. 11, 14, 125–145)

Type material.—*Holotype male.* AUSTRALIA: *Western Australia*: Grass Patch (IBRA_MAL), 33°14′S, 121°43′E, on toilet floor, 19 December 1995, B. & P. Starcevich (WAM T40696).

Paratypes. AUSTRALIA: *Western Australia*: 1 ♂, same locality data as holotype, 31 March 2005, C. & M. Longbottom (WAM T63207); 1 ♂, same locality data except grain bin area, 10 May 1987, J. Durbridge (WAM T26988); 1 ♂, same locality data except Grass Patch Primary School, on verandah, cool, wet, 8 May 1987, A.F. Longbottom (WAM T26987); 1 ♂, same locality data except Grass Patch townsite, on verandah at night, stormy, 28 March 2005, S. & D. Jacka (WAM T63206[DNA_Voucher_218]; GenB–CYB–MG652507, GenB–MRPL45–MG652546, GenB–RPF2–MG652565, GenB–XPNPEP3–MG652583, GenB–ITS–MG652518); 1 ♂, same data except on verandah in morning, 25 February 2001, A.F. Longbottom (WAM T65456); 1 ♀, same locality data except 'Sieda', dead on ground near shed, 3 December 1996, A.F. Longbottom (WAM T26986).

Other material examined.—AUSTRALIA: *Western Australia*: 1 ♂, Boorabbin Rock (IBRA_COO), 31°12′S, 120°17′E, pitfall trap, 1 March 1969, R. Jones (WAM T143058); 1 ♂, 8 km S. of Grass Patch, at Colemans (IBRA_MAL), 33°14′S, 121°43′E, hand collected at night on lawn, 22 December 1978, G. Finton (WAM T26983); 1 ♂, "The Glen", ca. 10 km W. of Grass Patch (IBRA_MAL), 33°14′S, 121°36′E, 25 February 2001, L. & C. Bowman (WAM T65457); 1 ♂, 300 m E. of railway line on Starcevich Road, ca. 2 km NE. of Grass Patch (IBRA_MAL), 33°13′09″S, 121°43′11″E, walking on road at night, 30 March 2005, A.F. Longbottom (WAM T63208); 1 ♂, McDermid Rock, site MRR6 (IBRA_COO), 32°01′00″S, 120°45′25″E, pitfall trap, *Eucalyptus salubris* woodland, 11–17 February 1981, W.F. Humphreys et al. (WAM T16190); 1 ♂, Red Lake (IBRA_MAL), 33°09′20″S, 121°42′45″E, climbing shade cloth at night, cool damp, 23 May 2006, L. Guest (WAM T76109[DNA_Voucher_217]; GenB–CYB–MG652508, GenB–MRPL45–MG652547, GenB–RPF2–MG652566, GenB–XPNPEP3–MG652584, GenB–ITS–MG652519); 1 ♂, same data except 33°08′S, 121°42′E, in toilet at farm, at night, 14 February 1979, M. Guest (WAM T26982); 1 juvenile, 5 km NNW. of Salmon Gums, on Coolgardie-Esperance Highway (IBRA_MAL), 32°56′22″S, 121°37′29″E, dug from burrow, 24 August 2014, S.E. Harrison, M.S. Harvey (WAM T134181[DNA_Voucher_66]; GenB–CO1–KY295313, GenB–CYB–KY295434, GenB–MRPL45–KY295554, GenB–RPF2–KY295680, GenB–XPNPEP3–KY295808, GenB–ITS–KY295061); 1 ♂, Yellowdine (IBRA_COO), 31°18′S, 119°39′E, hand collected, 21 January 1987, I. Land (WAM T29851).

Etymology.—This species is named in honor of Emeritus Professor Barbara Main (of the University of Western Australia), in recognition of her seminal contributions to mygalomorph systematics, and to our understanding of giant spiny trapdoor spiders of the genus *Gaius*.

Diagnosis.—Males of *Gaius mainae* can be distinguished from those of all other congeners except *G. aurora* by the absence of prolateral clasping spurs on tibia I (Figs. 132–133; cf. Figs. 23, 57, 79, 101, 114, 154); and from *G. aurora* by the size and shape of the RTA, which is very large (Fig. 134; cf. Fig. 46).

Description (male holotype).—Total length 31.0. Carapace 12.2 long, 10.5 wide. Abdomen 13.2 long, 8.6 wide. Carapace (Fig. 125) oval, dark chocolate-brown in color with mostly black ocular region; lateral margins densely setose; fovea slightly procurved. Eye group (Fig. 128) trapezoidal (anterior eye row strongly procurved), 0.7 x as long as wide, PLE–PLE/ALE–ALE ratio 1.5; ALE separated by 1.2 x their own diameter; AME separated by less than their own diameter; PME separated by 5.0 x their own diameter; PME and PLE separated by slightly more than 2.0 x diameter of PME, PME positioned in line with level of PLE. Maxillae with field of cuspules confined to inner corner; labium without cuspules. Abdomen (Fig. 126) oval, densely setose and dark grey-brown in dorsal view, with paler beige-brown mottling; sclerotized sigilla absent. Legs (Figs. 132, 133) variable shades of dark brown, with light scopulae on tarsi I–II; tibia I with pro-distal comb of macrosetae and row of numerous retro-ventral macrosetae; metatarsus I with 7 pro-ventral and 7 retro-ventral macrosetae. Leg I: femur 11.1; patella 5.3; tibia 7.3; metatarsus 7.5; tarsus 4.3; total 35.5. Leg I femur–tarsus/carapace length ratio 2.9. Pedipalpal tibia (Figs. 134–136) densely setose, 2.0 x longer than wide, with massive spinulate RTA and long, 'finger-like' distal retrolateral tibial apophysis (dRTA) also with sparse field of spinules. Cymbium (Figs. 134–136) setose, with field of weakly-developed spinules disto-dorsally. Embolus (Figs. 134–136) slightly twisted and gently tapered, with very short, triangular embolic apophysis sub-distally.

Description (female WAM T41380).—Total length 30.0. Carapace 14.2 long, 12.2 wide. Abdomen 10.7 long, 8.4 wide. Carapace (Fig. 137) broadly oval, dark tan-brown in color with mostly black ocular region; lateral margins densely setose; fovea procurved. Eye group (Fig. 140) trapezoidal (anterior eye row strongly procurved), 0.7 x as long as wide, PLE–PLE/ALE–ALE ratio 1.7; ALE separated by 2.0 x their own diameter; AME separated by approximately their own diameter; PME separated by 6.0 x their own diameter; PME and PLE separated by approximately 1.5 x diameter of PME, PME positioned in line with level of PLE. Maxillae with field of cuspules confined to inner corner; labium without cuspules. Abdomen (Fig. 138) oval, shriveled, brown in dorsal view;

sclerotized sigilla absent. Legs (Figs. 143, 144) variable shades of dark brown, with thick scopulae on tarsi and metatarsi I–II; tibia I with cluster of 8 pro-distal macrosetae and row of 6 long retro-ventral macrosetae; metatarsus I with 4 ventral macrosetae; ventral tarsus I with distal cluster of 4 short macrosetae. Leg I: femur 10.0; patella 5.9; tibia 5.9; metatarsus 5.5; tarsus 3.7; total 31.0. Leg I femur–tarsus/carapace length ratio 2.2. Pedipalp dark brown, spinose on tibia, with thick tarsal scopula. Genitalia (Fig. 145) with pair of large, widely spaced, mushroom-shaped spermathecae on broad stalks.

Distribution and remarks.—*Gaius mainae* (formerly known by WAM identification code 'MYG076') is a large species with a somewhat restricted distribution in the southern Coolgardie and eastern Mallee bioregions of southern inland Western Australia, from Southern Cross south to Grass Patch (Fig. 11). Males ($n = 14$) generally wander in search of females in the warm (mostly summer) months of December–March (79% of collected specimens), presumably after heavy rain, with a possible peak of activity in February and March (57% of collected specimens).

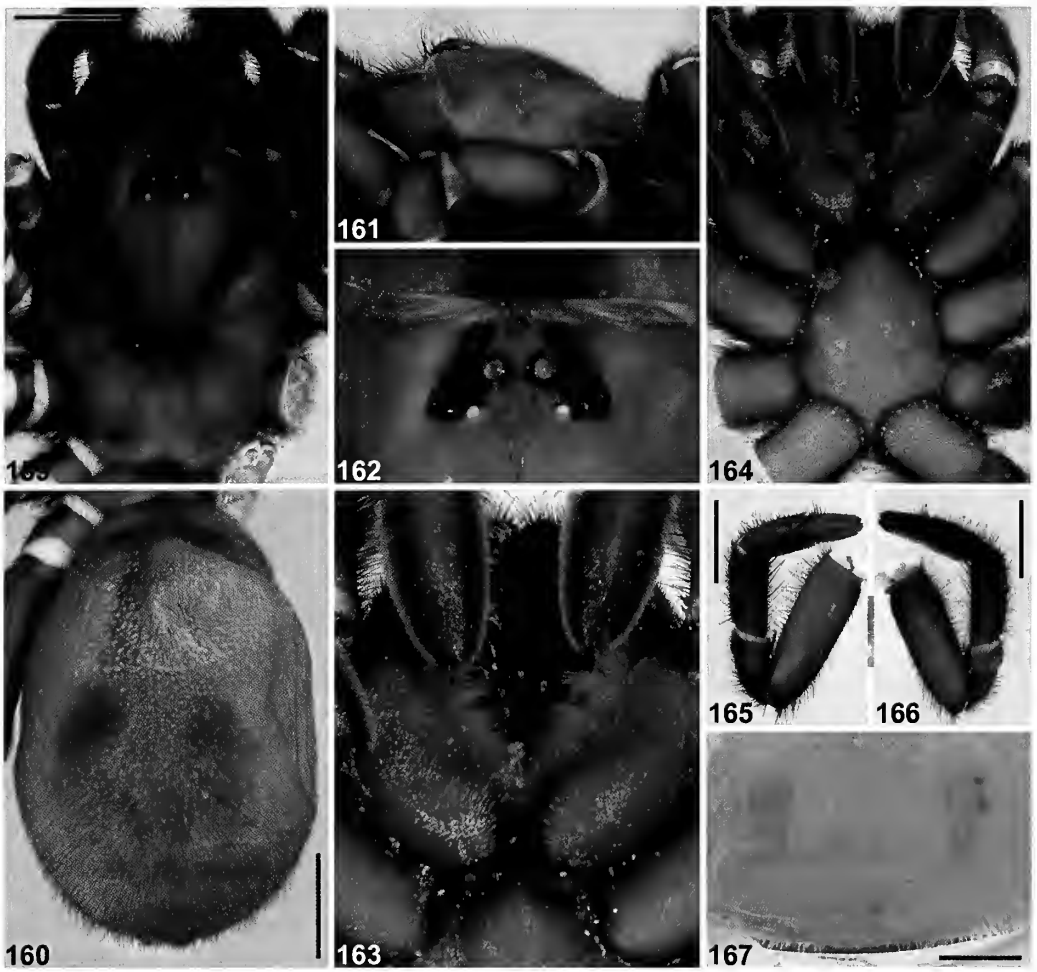
Conservation assessment.—This species has a known extent of occurrence (EOO) of approximately 20,000 km^2, and is therefore not considered threatened under Criterion B. However, preliminary evidence suggests that population declines may have occurred among arid zone Idiopidae in recent decades (Rix et al. 2017c), and given that the EOO of this species is approaching that which would render it potentially vulnerable under IUCN criteria, it is possible that future changes to EOO and area of occupancy may render this species threatened or near threatened. As a result, further ongoing assessment under both Criterion A and Criterion B is warranted.

Gaius tealei sp. nov.
http://zoobank.org/?lsid=urn:lsid:zoobank.org:act:5A32A02E-D2DB-4BC3-86F5-90E359110BBA
(Figs. 11, 14, 146–167)

Type material.—*Holotype male*. AUSTRALIA: *Western Australia*: Lorna Glen Station (IBRA_MUR), 26°16′21″S, 121°11′34″E, dry pitfall trap, 31 October 2011, K.E.C. Brennan (WAM T117550^{DNA_Voucher_223}; GenB–CYB–MG652503, GenB–MRPL45–MG652545, GenB–RPF2–MG652564, GenB–XPNPEP3–MG652577, GenB–ITS–MG652525).

Paratype. AUSTRALIA: *Western Australia*: 1 ♂, Lorna Glen Station, cat survey site 1-8 (IBRA_MUR), 26°18′12″S, 121°23′33″E, dry pitfall trap, 27 November 2004, M.A. Cowan et al. (WAM T66391).

Other material examined.—AUSTRALIA: *Western Australia*: 1 juvenile, Area C, 92.1 km NW. of Newman (IBRA_PIL), 23°00′37″S, 118°55′06″E, dug from burrow, 15 August 2010, R. Teale, J. Cairnes (WAM T105868^{DNA_Voucher_T105868}; GenB–COI–KJ744620); 1 juvenile, same data (WAM T105869^{DNA_Voucher_T105869}; GenB–COI–KJ744621); 1 ♀, same data except 23°00′38″S, 118°55′07″E, 16 August 2010 (WAM T105870^{DNA_Voucher_T105870}; GenB–COI–KJ744622); 1 juvenile, Area C, 98 km NW. of Newman (IBRA_PIL), 23°00′30″S, 118°51′13″E, dug from burrow, R. Teale, M. Greenham (WAM T103186^{DNA_Voucher_T103186}; GenB–COI–KJ744497); 1 ♀, Davidson Creek, ca. 75 km E. of Newman, site 9 (IBRA_GAS), hand collected, spinifex plain on gentle hill, *Acacia*, eucalypts, 9 April 2010, J. Clark (WAM T102164^{DNA_Voucher_290}; GenB–COI–KJ744437, GenB–CYB–MG652501, GenB–MRPL45–MG652544, GenB–RPF2–MG652562, GenB–XPNPEP3–MG652581, GenB–ITS–MG652523); 1 ♀, Fortescue Marsh (IBRA_PIL), 22°18′26.82″S, 119°14′26.40″E, 2–5 June 2010, P. Roberts, L. Quinn (WAM T107183^{DNA_Voucher_291}; GenB–COI–KJ744652, GenB–CYB–MG652502, GenB–MRPL45–MG652543, GenB–RPF2–MG652561, GenB–XPNPEP3–MG652580, GenB–ITS–MG652524); 1 ♂, Giles, ca. 55 km W. of Newman (IBRA_PIL), 23°15′S, 119°10′E, 23 December 1983 (WAM T27013); 1 ♂, Glen Ayle Homestead, 200 miles NE. of Wiluna (IBRA_GAS), 25°16′S, 122°02′E, 3 December 1975, W.K. Youngson (WAM T27014); 1 ♂, Glen Ayle Station (IBRA_GAS), 25°16′S, 122°03′E, 1999, T. Davis (WAM T143064); 1 ♀, Hope Downs 4, ca. 100 km NW. of Newman, HD4-8 (IBRA_PIL), 23°05′11.6″S, 119°19′06.1″E, 10 May 2008, J. Francesconi (WAM T91708^{DNA_Voucher_T91708}; GenB–COI–KJ745347); 1 ♀, same data except 12 May 2008 (WAM T91709^{DNA_Voucher_T91709}; GenB–COI–KJ745348); 1 ♀, Hope Downs 4, ca. 30 km NW. of Newman, HD4-11, 38-39 (IBRA_PIL), 23°06′05.22″S, 119°17′30.48″E, *Acacia* woodland over *Ptilotus* and *Triodia*, 24 September 2008, Francesconi Consulting (WAM T93466^{DNA_Voucher_T93466}; GenB–COI–KJ745379); 1 specimen (age unknown), same data except HD4-11, 40 (WAM T93464^{DNA_Voucher_T93464}; GenB–COI–KJ745377); 1 ♀, Jimblebar, 40 km ESE. of Newman (IBRA_PIL), 23°23′29.9″S, 120°06′34.4″E, 26 August 2008, C. Weston, G. Murray (WAM T92459^{DNA_Voucher_T92459}; GenB–COI–KJ745372); 1 specimen (age unknown), same data (WAM T92461^{DNA_Voucher_T92461}; GenB–COI–KJ745373); 1 ♀, same data except 22 August 2008 (WAM T92463^{DNA_Voucher_T92463}; GenB–COI–KJ745374); 1 ♀, Jimblebar, ca. 35 km E. of Newman (IBRA_PIL), 23°23′39″S, 120°11′58″E, 9 February 2009, P. Bolton, C. Weston (WAM T96024^{DNA_Voucher_T96024}; GenB–COI–KJ745411); 1 specimen (age unknown), Koodaideri Corridor West, 71.8 km NE. of Tom Price (IBRA_PIL), 22°08′08.3″S, 118°08′10.3″E, 20–26 February 2012, C. Cole (WAM T122239^{DNA_Voucher_T122239}; GenB–COI–KJ745001); 1 specimen (age unknown), same data except 22°08′09.9″S, 118°08′09.7″E (WAM T122249^{DNA_Voucher_T122249}; GenB–COI–KJ745005); 1 specimen (age unknown), same data except 72.4 km NE. of Tom Price, 22°08′08.4″S, 118°08′10.3″E (WAM T122240^{DNA_Voucher_T122240}; GenB–COI–KJ745002); 1 specimen (age unknown), same data except 82.5 km NE. of Tom Price, 22°13′56.8″S, 118°24′52.7″E (WAM T122253^{DNA_Voucher_T122253}; GenB–COI–KJ745009); 1 specimen (age unknown), same data except 82.4 km NE. of Tom Price, 22°13′57″S, 118°24′52.2″E, M. Greenham (WAM T122255^{DNA_Voucher_T122255}; GenB–COI–KJ745011); 1 specimen (age unknown), Koodaideri Western Corridor, 146.3 km NW. of Newman (IBRA_PIL), 22°21′28.7″S, 118°48′05.3″E, Mulga woodland, 2 April 2012, L. Alexander, J. Cairnes (WAM T125345^{DNA_Voucher_T125345}; GenB–COI–KJ745128); 1 juvenile, same data except 184.5 km NW. of Newman, 22°14′00.1″S, 118°23′47.4″E, low open Mulga woodland over *Triodia epactia* grassland, 31 March 2012, G. Humphreys, J.



Figures 159–167.—*Gaius tealei* sp. nov., female (WAM T127920) from 93 km E. of Marymia Airstrip (Western Australia; LSD): 159–160, carapace and abdomen, dorsal view; 161, cephalothorax, lateral view; 162, eyes, dorsal view; 163, mouthparts, ventral view; 164, cephalothorax, ventral view (note the presence of numerous presumably phoretic mites); 165, leg I, prolateral view; 166, leg I, retrolateral view; 167, spermatheeae, dorsal view. Scale bars = 5.0 (159–160, 165–166), 1.0 (167).

King (WAM T125333^{DNA_Voucher_T125333}; GenB–COI–KJ745120); 1 specimen (age unknown), same data except 185.3 km NW. of Newman, 22°14′00.2″S, 118°23′47.5″E (WAM T125334^{DNA_Voucher_T125334}; GenB–COI–KJ745121); 1 specimen (age unknown), same data except 185.6 km NW. of Newman, 22°14′00.38″S, 118°23′45″E, G. Humphreys, S. Werner (WAM T125331^{DNA_Voucher_T125331}; GenB–COI–KJ745119); 1 ♂, Leinster (IBRA_MUR), 27°55′S, 120°41′E, 15 February 1995, D. Murphy (WAM T143061); 1 ♀, 93 km E. of Marymia airstrip (IBRA_LSD), 25°08′22″S, 120°41′55″E, hand collected, ex. burrow with trapdoor lid & twig-lines, 66 cm deep, 16 August 2012, N.A. Guthrie (WAM T127920^{DNA_Voucher_29}; GenB–CYB–KY295471, GenB–MRPL45–KY295590, GenB–RPF2–KY295717, GenB–XPNPEP3–KY295845, GenB–ITS–KY295098); 1 specimen (age unknown), Mudlark, 102 km W. of Newman (IBRA_PIL), 23°05′25″S, 118°48′38″E, dug from burrow, 3 July 2011, C. Cole, N. Watson (WAM T116840^{DNA_Voucher_T116840}; GenB–COI–KJ744885); 1 specimen (age unknown), same data except 108 km W. of Newman, 23°02′33″S, 118°43′45″E, 2 July 2011, C. Cole, J. Cairnes (WAM T116789^{DNA_Voucher_T116789}; GenB–COI–KJ744866); 1 specimen (age unknown), same data except 112 km W. of Newman, 23°02′16″S, 118°40′56″E, 1 July 2011, M. Greenham, J. Cairnes (WAM T116766^{DNA_Voucher_T116766}; GenB–COI–KJ744853); 1 specimen (age unknown), same data except 113 km W. of Newman, 23°02′17″S, 118°40′57″E (WAM T116772^{DNA_Voucher_T116772}; GenB–COI–KJ744855); 1 specimen (age unknown), Murray Hill, Mulga Downs Station, Ecologia project 1142 (IBRA_PIL), 22°07′28.89″S, 118°31′04.98″E, 21 April–25 May 2009, N. Dight, L. Quinn (WAM T97639^{DNA_Voucher_T97639}; GenB–COI–KJ745447); 1 specimen (age unknown), same data (WAM T97640^{DNA_Voucher_T97640}; GenB–COI–KJ745448); 1 specimen (age unknown), 81.5 km NW. of Newman (IBRA_PIL), 23°01′24″S, 119°01′15″E, dug from burrow, 6 November 2011, M. Delaney (WAM T127197^{DNA_Voucher_T127197}; GenB–COI–KJ745171); 1 ♀, 111.6 km NW. of Newman (IBRA_PIL), 22°53′30″S, 118°45′50″E, dug from burrow, mulga woodland, 29 March 2012, N. Watson, P. Brooshooft (WAM T122819^{DNA_Voucher_T122819}; GenB–COI– KJ745047); 1 ♀, 117.2 km NW. of Newman (IBRA_PIL), 22°53′26″S, 118°42′22″E, dug from burrow, mulga woodland, 1 April 2012, C. Cole (WAM T122857^{DNA_Voucher_T122857}; GenB–

COI–KJ745081); 1 ♀, 118.6 km NW. of Newman (IBRA_PIL), 22°52′49″S, 118°41′16″E, dug from burrow, mulga woodland, 1 April 2012, P. Brooshooft (WAM T122868[DNA_Voucher_T122868]; GenB–COI–KJ745091); 1 ♀, 127.3 km NW. of Newman (IBRA_PIL), 22°51′37″S, 118°36′15″E, dug from burrow, mulga woodland, 29 March 2012, J. Tatler, N. Watson (WAM T122827[DNA_Voucher_T122827]; GenB–COI–KJ745055); 1 ♀, South Parmelia, 52 km NW. of Newman (IBRA_PIL), 23°05′12″S, 119°19′07″E, dug from burrow, 12 April 2011, R. Teale, M. Greenham (WAM T113577[DNA_Voucher_68]; GenB–COI– KJ744741, GenB–CYB–KY295432, GenB–MRPL45–KY295552, GenB–RPF2–KY295678, GenB–XPNPEP3–KY295806, GenB–ITS–KY295059); 1 specimen (age unknown), same data except 23°05′11″S, 119°19′07″E, 16 April 2011 (WAM T113592[DNA_Voucher_T113592]; GenB–COI–KJ744752); 1 ♀, West Angelas, 113 km SE. of Tom Price (IBRA_PIL), 23°19′09″S, 118°40′01″E, dug from burrow, 27 June 2010, E. Harris, C. Cole (WAM T103909[DNA_Voucher_T103909]; GenB–COI–KJ744574); 1 ♀, West Angelas, 31.6 km SE. of Juna Downs Homestead, site WES (IBRA_PIL), 23°05′06″S, 118°42′17″E, dug from burrow, 7 September 2005, R. Teale (WAM T77524[DNA_Voucher_T77524]; GenB–COI–KJ745258); 1 ♀, same data (WAM T77526[DNA_Voucher_T77526]; GenB–COI–KJ745259); 1 ♀, same data (WAM T77539[DNA_Voucher_T77539]; GenB–COI–KJ745264); 1 ♀, same data except 41 km SE. of Juna Downs Homestead, 23°06′01″S, 118°48′38″E, 10 September 2005 (WAM T77535[DNA_Voucher_T77535]; GenB–COI–KJ745262); 1 ♀, same data (WAM T77536[DNA_Voucher_T77536]; GenB–COI–KJ745263); 1 ♀, same data except 46.2 km ESE. of Juna Downs Homestead, 23°04′08″S, 118°53′24″E, 9 September 2005 (WAM T77532[DNA_Voucher_T77532]; GenB–COI–KJ745261); 1 ♀, same data (WAM T77543[DNA_Voucher_T77543]; GenB–COI–KJ745265); 1 ♀, same data (WAM T77544[DNA_Voucher_T77544]; GenB–COI–KJ745266); 1 ♂, Wiluna (IBRA_MUR), 26°35′S, 120°14′E, 1 March 1955 (WAM T27068); 1 ♂, same data (WAM T27069); 1 ♀, Wonmunna (IBRA_PIL), 23°08′02.14″S, 119°02′51.48″E, burrow excavation, 23 May–2 July 2011, J. Clark (WAM T112089[DNA_Voucher_312]; GenB–CYB–MG652500, GenB–MRPL45–MG652542, GenB–RPF2–MG652563, GenB–XPNPEP3–MG652579, GenB–ITS–MG652522); 1 ♂, Wonmunna, ca. 73 km heading 291° from Newman (IBRA_PIL), 23°07′06.78″S, 119°03′53.55″E, wet pitfall trap, south facing creek side slope, 20 May–22 June 2011, P.R. Langlands (WAM T115829); 1 ♂, Yandil Station (IBRA_MUR), 26°22′S, 119°49′E, hand collected, 7 February 1957, P. Molloy (WAM T3937); 1 ♂, same data (WAM T3938).

Etymology.—The specific epithet is named in honor of Roy Teale (of Biota Environmental Sciences), in recognition of his support of the Australian Research Council (ARC) idiopid project since its commencement in 2012, and for his considerable efforts in collecting Idiopidae from remote areas of Western Australia (including numerous specimens of this species).

Diagnosis.—Males of *Gaius tealei* can be distinguished from those of *G. aurora* and *G. mainae* by the presence of prolateral clasping spurs on tibia I (Figs. 153–155; cf. Figs. 45, 133); from *G. austini* and *G. humphreysi* by the size and shape of the RTA, which is grossly enlarged (Fig. 156; cf. Figs. 59, 116); and from *G. cooperi*, *G. hueyi* and *G. villosus* by the size and shape of the distal retrolateral tibial apophysis (dRTA), which is 'tongue-shaped' (Fig. 156; cf. Figs. 25, 81, 103).

Description (male holotype).—Total length 34.6. Carapace 12.8 long, 11.8 wide. Abdomen 15.8 long, 11.8 wide. Carapace (Fig. 146) broadly oval, dark chocolate-brown in color with mostly black ocular region; lateral margins densely setose; fovea slightly procurved. Eye group (Fig. 149) trapezoidal (anterior eye row strongly procurved), 0.6 x as long as wide, PLE–PLE/ALE–ALE ratio 1.6; ALE separated by 2.0 x their own diameter; AME separated by less than their own diameter; PME separated by 4.5 x diameter of right PME (left PME reduced); PME and PLE separated by slightly more than diameter of right PME, PME positioned slightly posterior to level of PLE. Maxillae with field of cuspules confined to inner corner; labium without cuspules. Abdomen (Fig. 147) oval, densely setose and grey-brown in dorsal view; sclerotized sigilla absent. Legs (Figs. 153–155) variable shades of dark brown, with light scopulae on tarsi I–II; tibia I with row of 5 long retro-ventral macrosetae and distal pair of prolateral clasping spurs; metatarsus I with 4 ventral and 5 retro-ventral macrosetae; tarsus I with 6 pro-ventral and 6 retro-ventral macrosetae. Leg I: femur 11.8; patella 5.6; tibia 8.6; metatarsus 7.9; tarsus 4.5; total 38.4. Leg I femur–tarsus/carapace length ratio 3.0. Pedipalpal tibia (Figs. 156–158) densely setose, 1.7 x longer than wide, with massive spinulate RTA and large, 'tongue-shaped' distal retrolateral tibial apophysis (dRTA) also with field of spinules. Cymbium (Figs. 156–158) setose, with field of weakly-developed spinules disto-dorsally. Embolus (Figs. 156–158) relatively broad at base, kinked medially and slightly twisted, with very short, triangular embolic apophysis sub-distally.

Description (female WAM T127920).—Total length 41.6. Carapace 15.0 long, 12.5 wide. Abdomen 21.4 long, 14.7 wide. Carapace (Fig. 159) oval, dark brown in color with mostly black ocular region; lateral margins densely setose; fovea procurved. Eye group (Fig. 162) trapezoidal (anterior eye row strongly procurved), 0.5 x as long as wide, PLE–PLE/ALE–ALE ratio 1.8; ALE separated by 2.9 x their own diameter; AME separated by approximately their own diameter; PME separated by 4.4 x their own diameter; PME and PLE separated by slightly more than 1.5 x diameter of PME, PME positioned slightly posterior to level of PLE. Maxillae with field of cuspules confined to inner corner; labium without cuspules. Abdomen (Fig. 160) oval, beige-brown in dorsal view; sclerotized sigilla absent. Legs (Figs. 165, 166) variable shades of dark brown, with thick scopulae on tarsi and metatarsi I–II; tibia I with cluster of 6 pro-distal macrosetae and row of 7 long retro-ventral macrosetae; metatarsus I with 1 pro-ventral and 4 retro-ventral macrosetae; ventral tarsus I with distal cluster of partially-obscured short macrosetae. Leg I: femur 9.4; patella 5.2; tibia 5.1; metatarsus 4.7; tarsus 3.3; total 27.7. Leg I femur–tarsus/carapace length ratio 1.8. Pedipalp dark brown, spinose on tibia, with thick tarsal scopula. Genitalia (Fig. 167) with pair of large, widely spaced spermathecae on long stalks.

Distribution and remarks.—*Gaius tealei* (formerly known by WAM identification codes 'MYG286' and 'MYG308') is a

very large species with a widespread distribution in northern inland Western Australia, from the Pilbara bioregion (mostly south of the Fortescue Marsh) south to Leinster and east to the Little Sandy Desert and eastern Gascoyne bioregion (Fig. 11). The range of this species overlaps that of *G. villosus* in the northern Murchison and southern Gascoyne bioregions, and also overlaps the range of *G. humphreysi* at its southern limit between Wiluna and Leinster. Males ($n = 9$) wander in search of females in the warm (mostly summer) months of December–March (78% of collected specimens), presumably after heavy monsoonal rain.

Conservation assessment.—This species has a known extent of occurrence of > 100,000 km^2, and is therefore not considered threatened under Criterion B. However, preliminary evidence suggests that population declines may have occurred among arid zone Idiopidae in recent decades (Rix et al. 2017c), and further assessment under Criterion A is warranted in the future.

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The identity of *Cercophonius himalayensis* Lourenço, 1996, and the exclusion of the scorpion family Bothriuridae from the Indian fauna

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Abstract. We studied the male holotype of *Cercophonius himalayensis* Lourenço, 1996, the sole member of the scorpion family Bothriuridae from India, and concluded that it belongs to a species of the genus *Phoniocercus* Pocock, 1893, which is endemic to the temperate forests of Patagonia. The presence of a Patagonian genus in India is extremely unlikely; therefore, we consider this to be a case of mislabeling of the specimen, and consequently exclude the scorpion family Bothriuridae from Indian fauna. *Cercophonius himalayensis* is transferred to the genus *Phoniocercus*, and formally synonymized with *Phoniocercus sanmartini* Cekalovic, 1968. A brief illustrated description of the type specimen is made, emphasizing important diagnostic characters and some body parts not previously described, such as the hemispermatophore. We also present a probable explanation for the origin of the material.

Keywords: *Phoniocercus*, Scorpiones, South America, Australia, Endemic, Gondwanaland

The scorpion family Bothriuridae comprises small to medium-sized fossorial species, mostly from temperate areas (Sissom 1990; Kovařík & Ojanguren-Affilastro 2013). This family is more diversified in southern and central South America with 14 genera and more than 140 species. It is also present in southern Africa with two basal genera and three species, as well as in Australia with one genus and six species (Acosta 1990; Prendini 2003a; Kovařík & Ojanguren-Affilastro 2013). The distribution of this family indicates a Gondwanic origin (Prendini 2003a). Maury (1975) suggested a paleo-Antarctic origin for Bothriuridae; however, at that time, he did not consider *Lisposoma* Lawrence, 1928 (the only African bothriurid genus known at the time) as a member of Bothriuridae.

Lourenço (1996) recorded the presence of a member of the family Bothriuridae in the Himalayas of India with the description of *Cercophonius himalayensis* Lourenço, 1996. Prior to that contribution, *Cercophonius* Peters, 1861 was only known from Australia (Koch 1977; Acosta 1990). The presence of a member of Bothriuridae in India is plausible, since this subcontinent was once part of Gondwana (Prendini 2003a); however, the absence of other bothriurid records from the Indian subcontinent, as well as the placement of the species in the genus *Cercophonius*, raised doubts about its identity. Although Bastawade et al. (2004) ignored this record in their identification key for the scorpions of India, Bastawade et al. (2012) later considered this species as part of the Indian fauna in their revision of the scorpion fauna of India. However, they followed the original description of Lourenço (1996) and did not contribute additional data about this species. In a taxonomic revision of family Bothiuridae, Kovařík & Ojanguren-Affilastro (2013) noticed that some of the characters in the original description of *C. himalayensis* (Lourenço 1996) did not fit with the diagnostic characteristics of the genus *Cercophonius*, and considered the species to be a *nomen dubium*. Kovařík & Ojanguren-Affilastro (2013) noted that a review of the holotype was fundamental to determine its identity. In a more recent revision of the scorpion fauna of India, Dupré (2017), also considered *C. himalayensis* as a *nomen dubium* and did not include it in his checklist of the Indian fauna. *Cercophonius himalayensis* is only known from the type specimen and to our knowledge, no members of the genus *Cercophonius* or family Bothriuridae have subsequently been reported from India or surrounding countries.

METHODS

Digital habitus images were taken under visible light, and images of external morphology under UV and visible light, using a Leica DFC290 digital camera attached to a Leica M165C stereomicroscope; the focal planes were fused with Helicon Focus 3.10.3 (online at http://heliconsoft.com). Scanning electron micrographs (SEM) were taken with a Philips XL30 TMP SEM at the Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" (MACN). Samples were dehydrated and coated with gold-palladium in a Thermo VG Scientific SC 7620 sputter coated prior to SEM.

Abbreviations for collections are as follows: MACN-Ar: Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", (Buenos Aires, Argentina); MNHN: Muséum National d'Histoire Naturelle, (Paris, France); ZMH: Zoologisches Museum, Hamburg, (Hamburg, Germany).

Descriptive terminology follows Mattoni & Acosta (2005) for hemispermatophores; Vachon (1974) for trichobothria; Francke (1977) for metasomal carinae, abbreviated as follows: DL: dorsolateral; LIM: lateral inframedian; LSM: lateral supramedian; LM: lateral median; VSM: ventral submedian; VL: ventrolateral; VM: ventromedian; and Prendini (2000) for pedipalp carinae, abbreviated as follows: DI: dorsal internal; DE: dorsal external; VI: ventral internal; VE: ventral external;

D: digital; E: external; IM: internomedian; EM: externomedian; V: ventral; VM: ventral median; DM: dorsal marginal; DS: dorsal secondary.

TAXONOMY

Family Bothriuridae Simon, 1880
Phoniocercus sanmartini Cekalovic, 1968
Figs. 1a–g, 2a–d, 3e–h; Table 1

Phoniocercus sanmartini Cekalovic 1968:64–73; Fet et al. 2000:38 (complete synonymic list until 1998); Prendini 2000:4, 5, 12, 19; Prendini 2003a:154; Prendini 2003b:242; Soleglad & Fet 2003:6; Fet et al. 2004:203; Rein 2007: 8; Kovařík & Ojanguren-Affilastro 2013:101; Ojanguren-Affilastro et al. 2013:114.

Cercophonius himalayensis Lourenço 1996:87–89. Kovařík 1998:101; Fet et al. 2000:33; Prendini 2003a:151; Rein 2007:8; Bastawade et al. 2012:4; Kovařík & Ojanguren-Affilastro 2013:74, 75 (*nomen dubium*); Dupre 2017:2. **New synonymy**.

Types examined.—*Phoniocercus sanmartini:* Holotype male, Lago Calafquen, Valdivia Province, Chile, 16 October 1964, Grupo de Ecólogos de la Facultad de Medicina Veterinaria de la Universidad de Chile (MZUC 455).

Cercophonius himalayensis: Holotype male, label data: India, Himalaya, Ukal, Pauri Garhwal, U.P., 30°N-79°5′E, about 45 km from the town of Pauri (2250 m alt.), 16 May 1958. F. Schmid (ZMH, Eing. Nr. A40/96).

Comparative diagnosis and taxonomic position of *Cercophonius himalayensis*.—The type specimen of *C. himalayensis* does not bear retrolateral pedal spurs on its legs (Fig. 1e), like all species of *Phoniocercus* Pocock, 1893 (Fig. 3e); however, *Cercophonius* bears well developed prolateral and retrolateral spurs (Fig. 3c). The spinal formula of telotarsi is: 3/3; ?/?; 4/4; 4/4; and the ventrolateral spines have setiform apices (Fig. 1e); the spinal formula of *Phoniocercus* is 3/3; 3/3; 4/4; 4/4, and also bears ventrolateral spines with setiform apices (Fig. 3e). The ventrolateral spines of *Cercophonius* species have blunt apices (Fig. 3c), with only the first one setiform, and the spinal formula is: 1/1; 2/2; 3/3; 3/3. The median ocelli are located in the proximal half of the carapace (Fig. 1a), as in *Phoniocercus*; in contrast to all *Cercophonius* species which have the median ocelli in the middle of the carapace. The dentate margin of the fingers of its pedipalp chelae bears only a single median row of denticles, which becomes double (or disordered) only in its basal part (Fig. 1h), and 5 pairs of internal and external accessory denticles, as in *Phoniocercus* (Fig. 3g), whereas in *Cercophonius* the dentate margin of the fingers bears 3 to 5 median rows of denticles, and 5 or 6 pairs of internal and external accessory denticles (Fig. 3b). Metasomal segment V has two VSM carinae extending along the whole segment and placed very close to the VM margin of the segment (Fig. 1g), as in *Phoniocercus* (Fig. 3h), whereas in *Cercophonius* the VSM carinae of metasomal segment V are reduced to some granules in the posterior third of the segment, joining with the VM carina (Fig. 3d). The telson vesicle is globose (Fig. 1c), as in males of *Phoniocercus*; being conspicuously less globose in *Cercophonius*. The hemispermatophore has a frontal crest at the base of the lamina (Fig. 2d), as in *Phoniocercus* (Fig. 3f), but not a frontal fold, nor two denticles in the external side of internal lobe as in *Cercophonius* (Fig. 3a). On the basis of these characteristics, this specimen is clearly not a member of genus *Cercophonius* but a species of *Phoniocercus*.

The distal lamina of the hemispermatophore is relatively short and blunt, and its frontal crest and distal crest are poorly developed, therefore this specimen should be assigned to *P. sanmartini*.

The measurements of the holotype of *C. himalayensis*, a male specimen of *C. michaelseni* Kraepelin, 1908, and a male specimen of *P. sanmartini* are compared in Table 1.

Redescription of *C. himalayensis* holotype.—*Color:* Base color reddish brown, with a variegated pattern in carapace, legs, tergites, sternites, and metasoma; however, in most parts of the body, this pattern is faded (presumably owing to poor preservation). Tergites with 2 lateral spots, separated by a median, incomplete and unpigmented stripe in the anterior two thirds of the segment, both lateral spots connected by reticular pigment in the posterior third of the segment. Sternites, legs and carapace with reticulated pattern. Metasoma: Ventral surface, segments I–IV with 3 stripes, 2 VL which are narrow anteriorly and become wider posteriorly, and 1 VSM sub-triangular spot with its anterior angle in the anterior margin of the segment and the posterior angles connecting with the posterior third of the VL stripes; segment V ventrally with 2 VL wide stripes, and a thin VM stripe. Dorsal surface: Segments I–III each with a median triangular spot, and posterolateral reticulate pigment; segment IV with posterolateral reticulate pigment; segment V with faint pigment on the dorsolateral margins. Telson: Ventral surface of the vesicle with faint pigment pattern; dorsal surface whitish due to the presence of a glandular area; reddish aculeus.

Carapace: Anterior margin with a conspicuous median notch that divides the anterior margin into 2 lobes (Fig. 1a). Surface smooth. Anteromedian longitudinal sulcus, interocular sulcus; posteromedian longitudinal and posterolateral sulci present and conspicuous, but not very deep. Median ocular tubercle small, placed slightly proximal from the middle of the carapace; median ocelli well developed, 2 diameters apart. Three small lateral ocelli on each side of the anterior margin of the carapace, 2 larger anterior ocelli in the same horizontal axis, and 1 smaller posterior ocellus situated slightly dorsal to the others; lateral ocelli pattern type 3A (Loria & Prendini 2014).

Chelicerae: Movable finger, distal internal tooth well developed, strongly curved, forming an angle of almost 90° with the rest of the finger, and slightly displaced ventrally with respect to the line formed by the other teeth; with 2 well-developed subdistal teeth (Fig. 2c).

Pedipalps: Femur, surface smooth; VE carina absent; DE, DI and VI carinae granular and well-developed, extending the entire length of the segment. Patella DI, VI, and VE carinae, extending the entire length of the segment, DI represented by some scattered big granules, VI carina poorly developed. Chela manus slender; with barely discernible carinae, with a small conical apophysis near the base of the movable finger (Fig. 1f), that partially surrounds a small internal smooth depression; with a small group of granules near the base of fixed finger, but not joining the granules of the dentate margin;

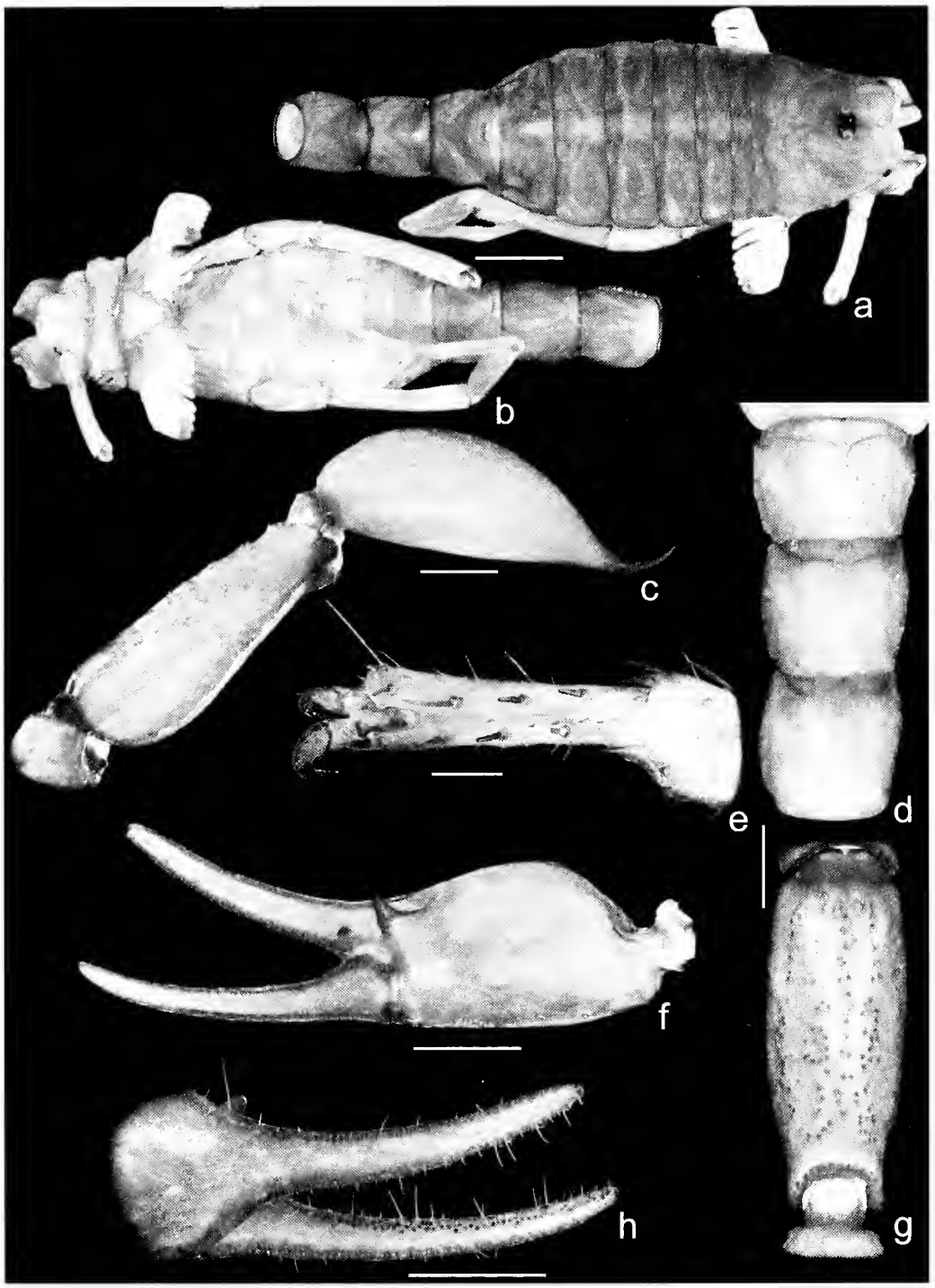

Figure 1.—*Cercophonius himalayensis*, holotype male: a. Habitus, dorsal aspect under visible light; b. Habitus, ventral aspect under visible light; c. Metasomal segment V and telson, lateral aspect under UV light; d. Metasomal segments I–III, ventral surface, under UV light; e. Telotarsus III, ventral surface under visible light; f. Pedipalp chela, ventrointernal surface under visible light; g. Metasomal segment V, ventral surface under UV light; h. Pedipalp chela, detail of fingers from dorsal view under visible light. Scale bars: a, b: 0.2 mm; c–h: 1 mm.

fingers medium-sized and slightly curved, dentate margin of fingers with a single row of denticles, and 5 pairs of internal and external accessory denticles, the basal external accessory granule forming part of the median row, the median row is quite disordered basally, becoming double in some areas (Fig. 1h). Trichobothrial pattern neobothriotaxic major Type C, with one accessory trichobothrium in *V* series of chela; femur with 3 trichobothria (*d*, *i*, *e*), one macroseta (M1) associated with *d* and *i*, *e* situated slightly proximal to M1; patella, with 19 trichobothria (2 *d*, *i*, 3 *et*, *est*, 2 *em*, 2 *esb*, 5 *eb*, 3 *V*); chela with 27 trichobothria (*Dt*, *Db*, 5 *Et*, *Est*, *Esb*, 3 *Eb*, *dt*, *dst*, *dsb*, *db*, *et*, *est*, *esb*, *eb*, *ib*, *it*, 5 *V*), V_2 displaced externally with respect to the line of *V* trichobothria; *Esb* forming a triangle with Eb_2 and Eb_3.

Table 1.—Measurements in mm of the holotype male of *Cercophonius himalayensis*, a male specimen of *Phoniocercus sanmartini* (Chiloe Island, Chile), and a male of *Cercophonius michaelseni* (taken from Acosta 1990).

	Cercophonius himalayensis, holotype male	*Phoniocercus sanmartini*, male	*Cercophonius michaelseni*, male
Total length	24.20	29.82	23.60
Carapace, length	2.93	3.53	2.99
Carapace, anterior width	2.00	2.26	1.83
Carapace, posterior width	3.46	3.80	3.23
Mesosoma, total length	5.66	8.11	5.11
Metasoma, total length	15.61	18.18	15.50
Metasomal segment I, length	1.33	1.66	1.39
Metasomal segment I, width	2.06	2.20	1.71
Metasomal segment II, length	1.73	2.00	1.71
Metasomal segment II, width	1.86	2.00	1.59
Metasomal segment III, length	1.93	2.33	1.83
Metasomal segment III, width	1.80	1.90	1.52
Metasomal segment IV, length	2.20	2.53	2.27
Metasomal segment IV, width	1.66	1.86	1.44
Metasomal segment V, length	3.66	4.06	3.71
Metasomal segment V, width	1.73	1.93	1.36
Metasomal segment V, height	1.46	1.60	1.24
Telson, length	4.76	5.60	4.59
Vesicle, length	3.93	4.60	3.39
Vesicle, width	1.86	2.10	1.39
Vesicle, height	1.60	1.66	1.12
Aculeus, length	0.83	1.00	1.20
Femur, length	2.86	3.33	2.87
Femur, width	0.93	1.00	0.84
Patella, length	3.20	3.66	2.91
Patella, width	1.06	1.13	0.99
Chela, length	5.35	6.26	5.19
Chela, width	1.60	1.66	1.39
Chela, height	1.60	1.86	1.24
Movable finger, length	3.20	3.40	3.07

Legs: Basitarsi with medium-sized prolateral spurs, but without retrolateral pedal spurs (Fig. 1e). Telotasi: ungues well developed and strongly curved, with ventrointernal and ventroexternal spines, each ending in a seta-shaped tip; since the material is poorly preserved most of the tips of these spines are broken, and spinal apices are generally blunt. Spinal formula leg I: 3/3, with the basal internal spine setiform; leg II: unknown (lost); legs III and IV: 4/4. Almost all legs are detached from the body, only right leg IV is still attached; both legs II, and left leg III are lost.

Sternum: Barely visible, strongly compressed longitudinally.

*Genital operc*ula: Sclerites subtriangular, strongly enlarged posteriorly (Fig. 1b).

Pectines: With 1 row of median lamellae. Fulcra present, small. Pectinal teeth large; tooth count: 11–11, but one tooth is missing in each pecten.

Tergites: Tergites I–VI, surfaces smooth; VII with paired submedian carinae and lateral carinae; paired submedian carinae are restricted to posterior third of segment, and lateral carinae are restricted to posterior half; intercarinal surfaces smooth.

Sternites: Sternites III–VII, surfaces entirely smooth; III–VI each with small, elliptical spiracles.

Metasoma: Metasomal segments I–III: DL and LSM carinae well developed, LIM carina poorly developed becoming less developed in posterior segments; VL and VSM carinae poorly developed, only represented as a slight elevation of the tegument, becoming more developed in posterior segments (Fig. 1d). Metasomal segment IV: DL and LSM carinae well developed, LIM carina absent, VL and VSM carinae well developed. Metasomal segment V: DL carina well developed, LSM and LIM carinae absent, lateral margins granular, VL carinae poorly developed and displaced externally almost to the lateral margins; VSM carinae granular, subparallel, extending the entire length of the segment, placed very close to the VM carina (Fig. 1g); VM carina barely visible, represented by some granules in the median part of the segment.

Telson: Vesicle globose dorsal surface smooth, slightly concave, telson gland not very conspicuous; ventral surface slightly granular. Aculeus very short, moderately curved (Fig. 1c).

Hemispermatophore: Distal lamina well developed, slightly curved, and similar in length to the basal portion; distal crest almost straight, occupying the apical fourth of the distal lamina, without transversal keels. Frontal crest small, forming a sub-circular distal lobe in the basal portion of the distal lamina (Figs. 2b, d). Lobe region was not examined because we could not clean it from surrounding tissues due to the poor condition of the material (Fig. 2a).

Remarks.—The specimen is poorly preserved; several body parts are broken and separated from the body, and some of them even missing (Figs. 1a, b).

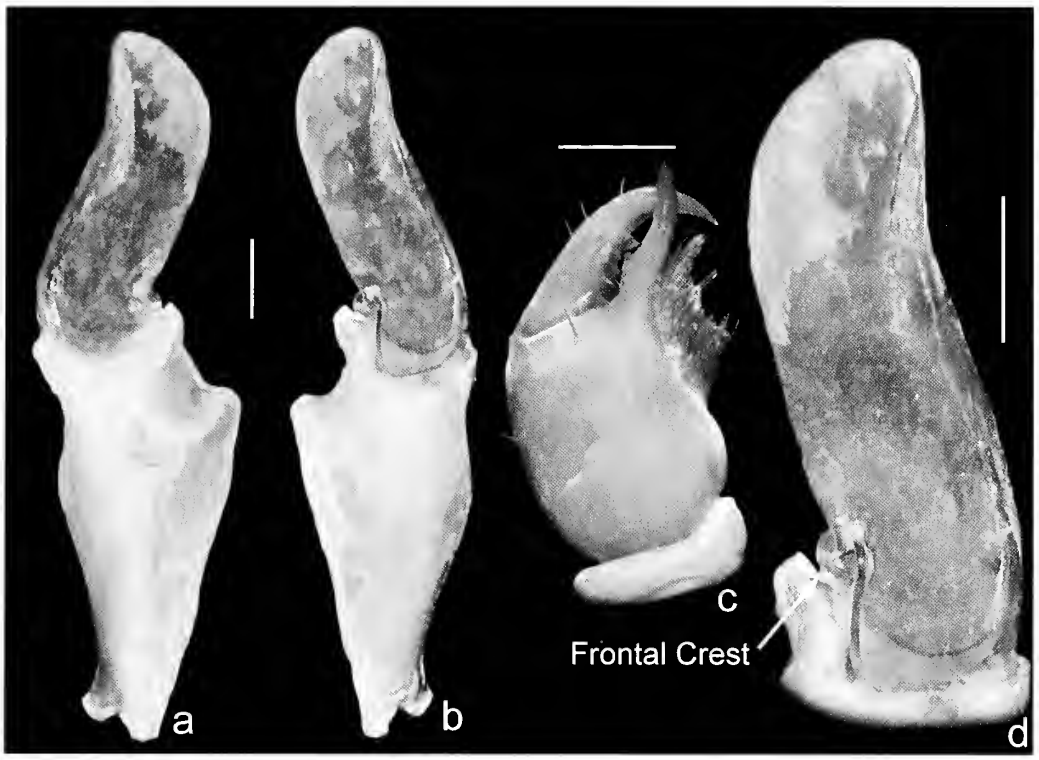

Figure 2.—*Cercophonius himalayensis*, holotype male: a. Left hemispermatophore, internal surface under visible light; b. Left hemispermatophore, external surface under visible light; c. Left chelicera, dorsal aspect under UV light; d. Detail of distal lamina of hemispermatophore, external aspect under UV light. Scale bars: 0.5 mm.

The genus *Phoniocercus* includes only two described species: *P. pictus* Pocock, 1893, the type species of the genus, and *P. sanmartini*. Both species share several characters; the females are extremely similar, but males can be separated by characteristics of the hemispermatophore: in *P. pictus* the distal lamina is more elongated and narrower than in *P. sanmartini*. In addition, the frontal crest and the distal crest are both more developed in *P. pictus* than in *P. sanmartini* (see Figs. 2b, 2d, 3f, and San Martín & Cekalovic (1968) for drawings of *P. pictus*, and Cekalovic (1968) for *P. sanmartini* description). Using these characters, we assigned the type specimen of *C. himalayensis* to *P. sanmartini* and here synonymize *C. himalayensis* with *P. sanmartini*. This species inhabits humid, evergreen forest in the Región de Los Ríos (XIV) and Región de Los Lagos (X), in the extreme south of Chile (Cekalovic 1968).

DISCUSSION

The labels, history and locality of *Cercophonius himalayensis*.—At the time of our examination of the specimen, the holotype was accompanied by six different labels (Fig. 4). These labels were assigned to three different authors/origins on the basis of handwriting characteristics. Two of these labels belong to the Zoologisches Museum of Hamburg, and are printed with that inscription; one contains all the information of the locality, collectors and collection date of the specimen, whereas the other corresponds to the identification of the specimen as the type material of *C. himalayensis*, as well as the author of the species. A second group of three labels were probably written by the author of the species; one includes the identification of the specimen as the type material of *C. himalayensis*, another one includes the collector and year of collection, and the remaining refers to "other data in the paper." Finally, there is a single label corresponding to Lorenzo Prendini who revised the material in 2004 and determined it as the holotype of *C. himalayensis*. After considering the information already noted in this contribution, we conclude that none of these labels is the original label/s of this specimen.

According to Lourenço (1996) the specimen was loaned to Dr. Max Vachon at the MNHN of Paris in the 1970s. Vachon apparently recognized it as a member of Bothriuridae, and then returned it to the ZMH, never publishing his discovery. Many years later Lourenço was able to locate the specimen, confirmed the decision of Vachon, and published his description of the species (Lourenço 1996).

Scorpions of the genus *Phoniocercus* are climax species of wet Patagonian forests. The presence of an introduced (exotic) population of *Phoniocercus* in India is unlikely, and the possibility that a single specimen came into India with imported goods from Chile is remote. Bothriuridae cannot be considered part of the Indian fauna with the data currently available.

We consider that the most plausible explanation for the labels of this specimen is that the specimen was mislabeled at some point of its history, or the tubes containing the specimen inter-changed between some Indian and Chilean material. Supporting our hypothesis of a mislabeled specimen, the "collector", the entomologist Fernand Schmid (1924–1998),

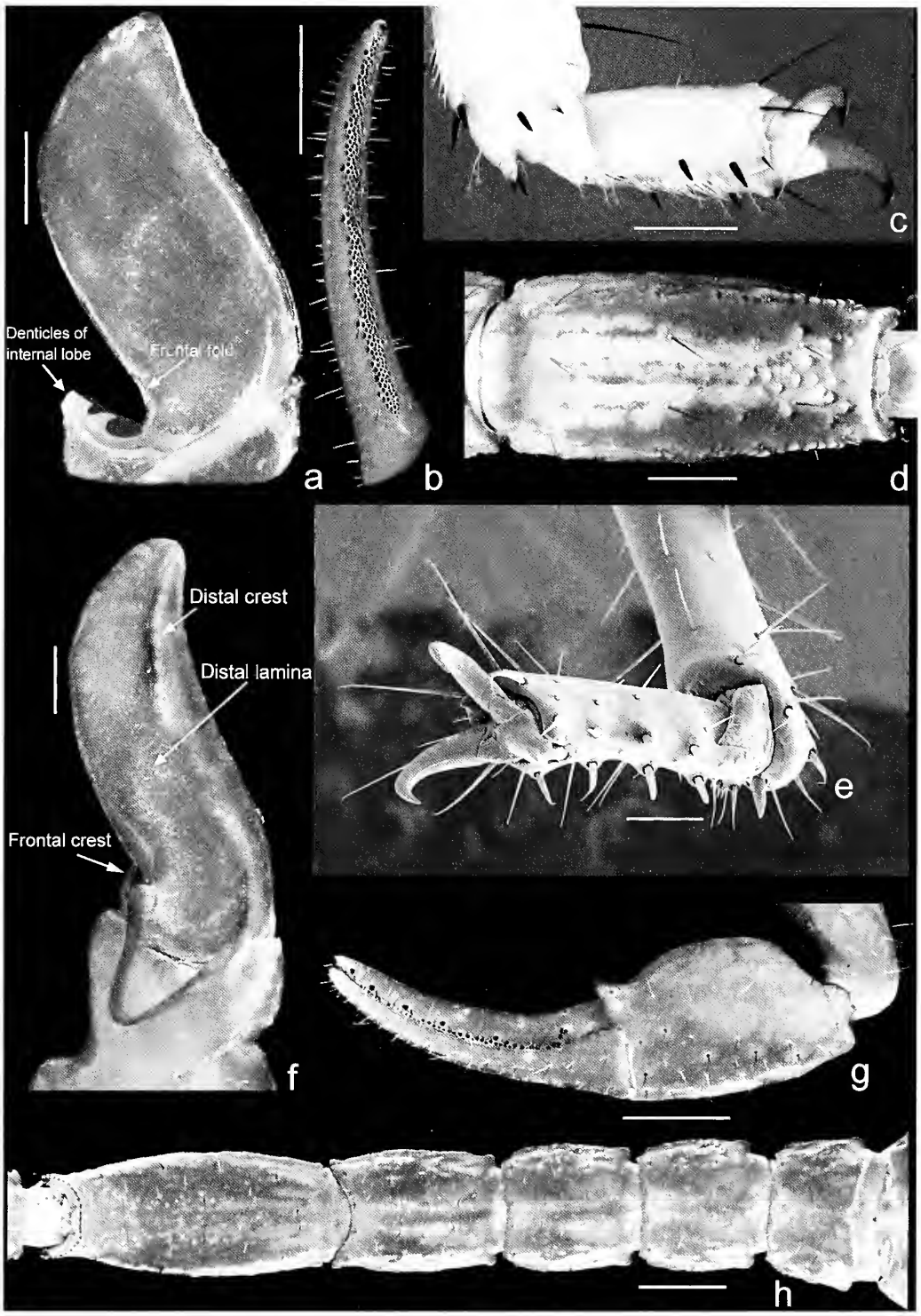

Figure 3.—*Cercophonius michaelseni*, male (from Tammin, Western Australia, MACN-Ar): a. Detail of distal lamina of hemispermatophore, external aspect under UV light; b. Detail of the internal surface of the movable finger of left pedipalp chela under UV light; c. Detail of the ventral surface of telotarsus III under UV light; d. Metasomal segment V, ventral surface, under UV light. e–h. *Phoniocercus sanmartini*, male (from Chacao, Chiloe Island, Chile, MACN-Ar): e. SEM image of ventral surface of telotarsus IV; f. Detail of distal lamina of hemispermatophore, external aspect under UV light; g. Ventrointernal surface of right pedipalp chela under UV light; h. Ventral surface of metasomal segments under UV light. Scale bars: a, c, e, f: 0.3 mm; b, d, g, h: 1 mm.

was actually collecting in Uttar Pradesh (India) in 1958 (Weaver & Nimmo 1999). At the same time, he was also working with material collected in Chile (Schmid 1958) by Luis Enrique Peña (1921–1995). Peña was a Chilean entomologist who regularly sent arachnid and insect specimens from Chile to researchers, museums and collections around the world (Barriga-Tuñón & Ugarte-Peña 1995). It is plausible that at some time, one of the labels of F. Schmid was placed

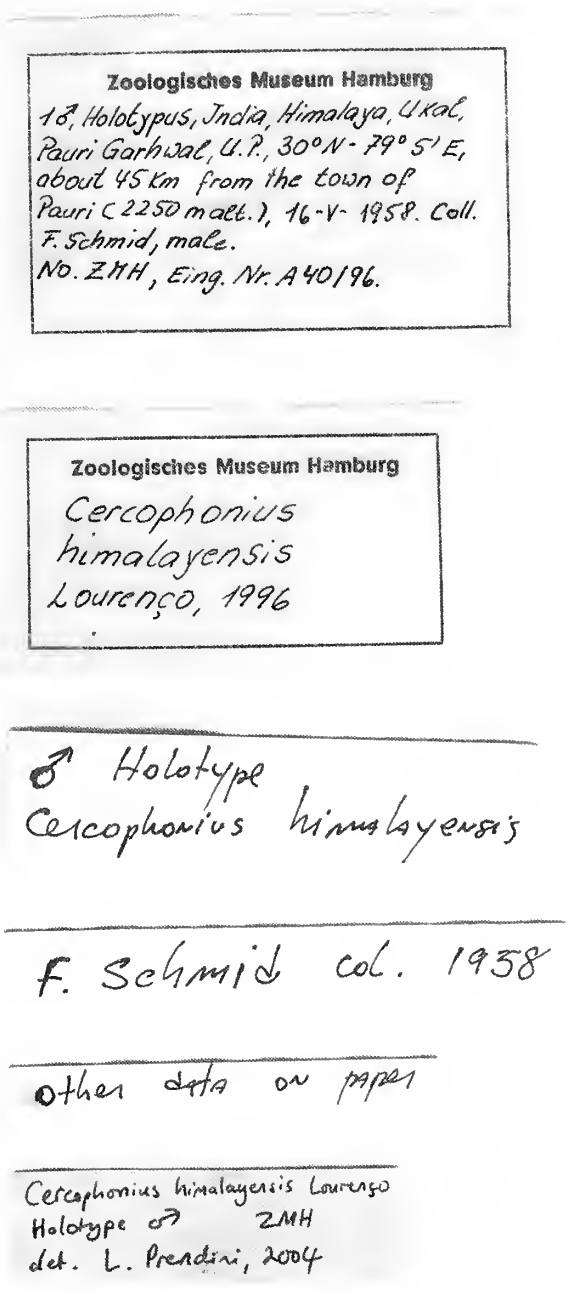

Figure 4.—Scanned image of all the labels currently accompanying the type material of *Cercophonius himalayensis*.

inside the vial of the scorpion collected by L.E. Peña, that ended in the Zoologisches Museum of Hamburg.

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Monophyly of the subfamily Neobisiinae (Pseudoscorpiones: Neobisiidae)

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Abstract. Members of the Neobisiidae are currently classified in two subfamilies, Neobisiinae and Microcreagrinae. Taxonomic assignment to subfamily is based upon two morphological characters, neither of which is consistently found within either subfamily. The form of the galeae is elongate and hyaline in the Microcreagrinae, but reduced and sclerotic in the Neobisiinae. However, some members of the Microcreagrinae also have reduced galeae. The position of trichobothrium *ist* located on the fixed finger of the pedipalp chelae is generally positioned subdistally and closer to trichobothrium *est* in Neobisiinae but sub-basally and closer to trichobothrium *ib* in Microcreagrinae. However, members of the genus *Parobisium*, currently assigned to the subfamily Neobisiinae, have a microcreagrine-like subbasal trichobothrium *ist*. Since neither subfamily is defined by an undisputed apomorphy, the monophyly of both groups has long been questioned. In this study, we tested whether or not the two subfamilies are monophyletic by inferring the phylogeny of the family using DNA sequence data from the mitochondrial protein-coding gene, COI, and the nuclear ribosomal gene 28S. Results of the molecular phylogenetic analyses indicate that neither of the subfamilies is monophyletic as presently defined. We transfer the genus *Parobisium* to the Microcreagrinae in order to simultaneously obtain a monophyletic Neobisiinae and resolve character inconsistency for the position of trichobothrium *ist*, which is sub-distal in all Neobisiinae taxa included in our study. We also find that reduction of the galea is not a reliable character state at the subfamily level, and has occurred at least three times independently within the family.

Keywords: Galea, molecular systematics, trichobothriotaxy, Pseudoscorpiones

Pseudoscorpion adults and nymphs spin silken chambers using their galeae, or spinnerets, present on the movable finger of each chelicera, which connect to silk glands in the cephalothorax (Chamberlin 1931). Most pseudoscorpions spin silken chambers for molting or brooding, and some species also build them for periods of quiescence, such as hibernation or aestivation (Kew 1914). Other species have more specialized uses for silken chambers. For example, *Lasiochernes pilosus* (Ellingsen, 1910) uses them as retreats to which they return after foraging, and therefore leave an opening through which they can enter and exit (Weygoldt 1969). *Halobisium occidentale* Beier, 1931 lives in the mud of marshes and estuaries and uses the silken chambers to create a dry space to live in (Lee 2007). Regardless of the function, the general shape of silken chambers appears to be very similar throughout the order, but there is a great diversity in the shape of the galeae among lineages (Kew 1914).

Differences in galeae shape comprise one of only two characters currently used to distinguish between members of two neobisiid subfamilies, the Neobisiinae Chamberlin, 1930 and Microcreagrinae Balzan, 1892. Members of the Microcreagrinae usually have elongate galeae, whereas members of the Neobisiinae have reduced galeae (Chamberlin 1930; Beier 1932; Harvey 1992). However, the stability and taxonomic utility of this character has been questioned (Murienne et al. 2008; Zaragoza 2008) as there are several microcreagrines that have neobisiine-like, reduced galeae (e.g., *Roncocreagris iglesiasae* Zaragoza, 2003; *R. murphyorum* Judson, 1992; *R. galeonuda* (Beier, 1955); *R. clavata* (Beier, 1955); and *R. robustior* (Beier, 1959)).

The only other character used to distinguish between these two subfamilies is a difference in the position of the trichobothrium *ist*, on the prolateral surface of the fixed finger of the pedipalp chelae. Harvey & Volschenk (2007) summarized the position of trichobothrium *ist* across the genera of Neobisiidae. Their work reveals that, in general, most microcreagrines have *ist* subbasal (Fig. 1c), whereas most neobisiines have *ist* subdistal (Fig. 1a). However, there are also exceptions for this character. For example, *Acanthocreagris* Mahnert, 1974 and *Insulocreagris* Ćurčić, 1987 are microcreagrines with *ist* subdistal, and *Parobisium* Chamberlin, 1930, *Occitanobisium* Heurtault, 1977, and *Trisetobisium* Ćurčić, 1982 are neobisiines with *ist* subbasal.

Not surprisingly for groups that lack defining apomorphies, the monophyly of both subfamilies have long been questioned (Vachon 1946; Mahnert 1974; Zaragoza 2008; Judson 2013). A molecular phylogenetic analysis of the entire order Pseudoscorpiones (Murienne et al. 2008) provided further indication that the Microcreagrinae may not be monophyletic, although only a few neobisiid genera were included. Here, we build upon this work to test whether the subfamilies are monophyletic. In particular, we explore the placement of the genus *Parobisium*, currently assigned to the subfamily Neobisiinae, since species in this genus have reduced galea but also have trichobothrium *ist* placed in a subbasal position (Chamberlin 1930).

METHODS

Neobisiid specimens were freshly collected by GBH, donated by other collectors, or obtained from the collection at the Museum of Comparative Zoology (MCZ) at Harvard University. Sequences of non-neobisiid taxa for the molecular phylogeny were primarily obtained from the NCBI GenBank database. In total, we analyzed COI and 28S sequences from 56 specimens representing 42 species of neobisiids and 9 outgroup taxa (Table 1).

DNA extractions were performed with the Qiagen DNEasy Blood & Tissue kit following the standard ATL buffer protocol for extraction from tissues. For each specimen,

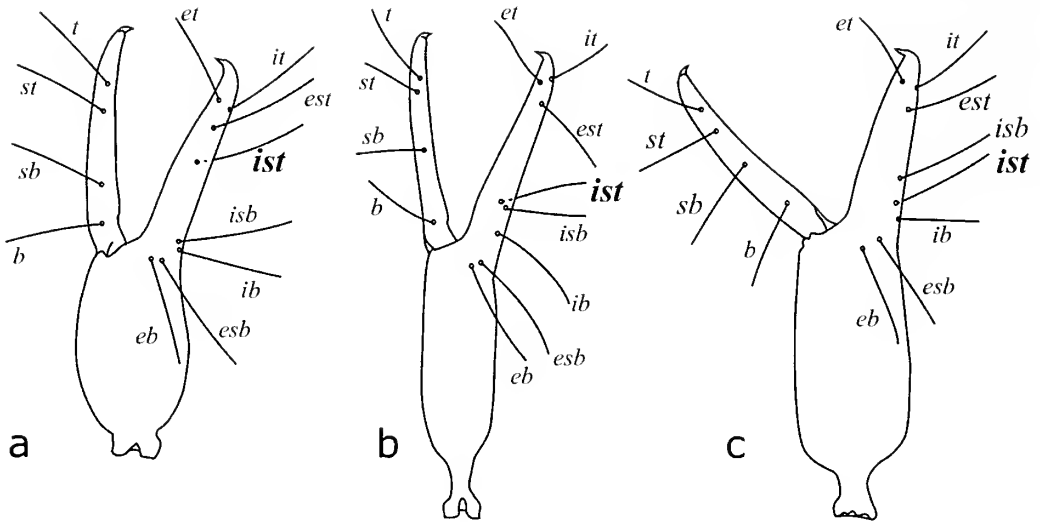

Figure 1.—External aspect of pedipalp chelae, illustrating the different positions of trichobothrium *ist*: The *ist* is subdistal and nearest to trichobothrium *est* (a) or subbasal and nearest trichobothrium *isb* (b and c). a. *Novobisium carolinense*, b. *Parobisium charlotteae*, c. *Americocreagris columbiana*. Illustrations adapted from Chamberlin (1962).

DNA was extracted either from the chelae or from the whole body. For extractions from the chelae, the chelae were dissected from the rest of the body and placed directly in a Proteinase K solution. For the whole body extractions before placing specimens in a Proteinase K solution, we removed the pedipalps and punctured the pleural membrane of the abdomen to facilitate access of the protein-degrading solution to the internal soft tissues. Voucher specimens for this project are deposited in the MCZ, the University of Arizona Insect Collection (UAIC), and the Muséum National d'Histoire Naturelle, Paris (MNHN) as indicated in Table 1.

DNA was amplified using Eppendorf Mastercycler model 5333 or Eppendorf Mastercycler gradient model 5331 (Eppendorf, Hamburg, Germany). Primers used for cytochrome oxidase subunit 1 (COI) were the forward primer LCO1490 (GCATAGTTCACCATCTTTC) and the reverse primer HCO2198 (TAAACTTCAGGGTGACCAAAAAATCA). Primers for 28S ribosomal DNA (28S) included the forward primers LS30F (ACCCCCTRAATTTAAGCATAT) and LS58F (GGGAGGAAAAGAAACTAAC), and the reverse primers and LS1066R (CGACCGATTTGCACGTCAG) and LS1126R (TCGGAAGGAACCAGCTACTA). For all genes, the PCR protocol included an initial temperature of 94°C for 2 minutes, followed by 35 cycles of denaturing at 94°C for 30 seconds, annealing at 50–55°C for 30 seconds, and extension at 72°C for 60–95 seconds. PCR products were cleaned, quantified, normalized and sequenced in both directions at the University of Arizona's Genomic and Technology Core Facility using a 3730 or 3730XL Applied Biosystems automatic sequencer. Chromatograms were assembled and initial base calls were made for each gene with Phred (Green & Ewing 2002) and Phrap (Green 1999) as orchestrated by Mesquite Ver. 3.4 (Maddison & Maddison 2018) and Chromaseq vers. 1.3 (Maddison & Maddison 2017). Final base calls were made in Mesquite and ambiguous bases were designated by a standard ambiguity code.

Sequences were aligned with MAFFT v.7.310 (Katoh & Standley 2013) within Mesquite using the E-INS-I setting for the 28S sequences and default settings for COI. The aligned matrix was partitioned by gene and by codon position, with each partition allowed to have independent parameter values for the model of evolution. Maximum likelihood (ML) inference was conducted using 500 heuristic searches RAxML 8.0.9 (Stamatakis 2014) under the GTR+gamma model of evolution on CIPRES Science Gateway portal (Miller et al. 2010). Clade support was conducted using rapid bootstrapping with a subsequent ML search and letting RAxML halt bootstrapping automatically (using MRE-based bootstopping criterion).

RESULTS

The optimal tree resulting from maximum likelihood analysis of the concatenated matrix is presented in Fig. 2b and the majority-rule consensus trees for the bootstrap analyses are presented for the concatenated matrix (Fig. 2a), the COI matrix (Fig. 3a), and the 28S matrix (Fig. 3b). In all analyses, Microcreagrinae was recovered as paraphyletic and Neobisiinae (minus *Parobisium*) formed a well-supported clade.

Our phylogeny implies three independent reductions of the galeae within the family (see black dots on Fig. 2b) and that trichobothrium *ist* is subdistal only in the Neobisiinae.

DISCUSSION

That the Microcreagrinae was recovered as paraphyletic in our analysis is not surprising given the number of authors who have commented on the heterogeneity of this subfamily and the potential for paraphyly (e.g., Vachon 1946; Mahnert 1974; Murienne et al. 2008; Zaragoza 2008). Several North American genera erected in the 1980s remain poorly diagnosed, making placement of new species into those genera problematic (Harvey & Muchmore 2010).

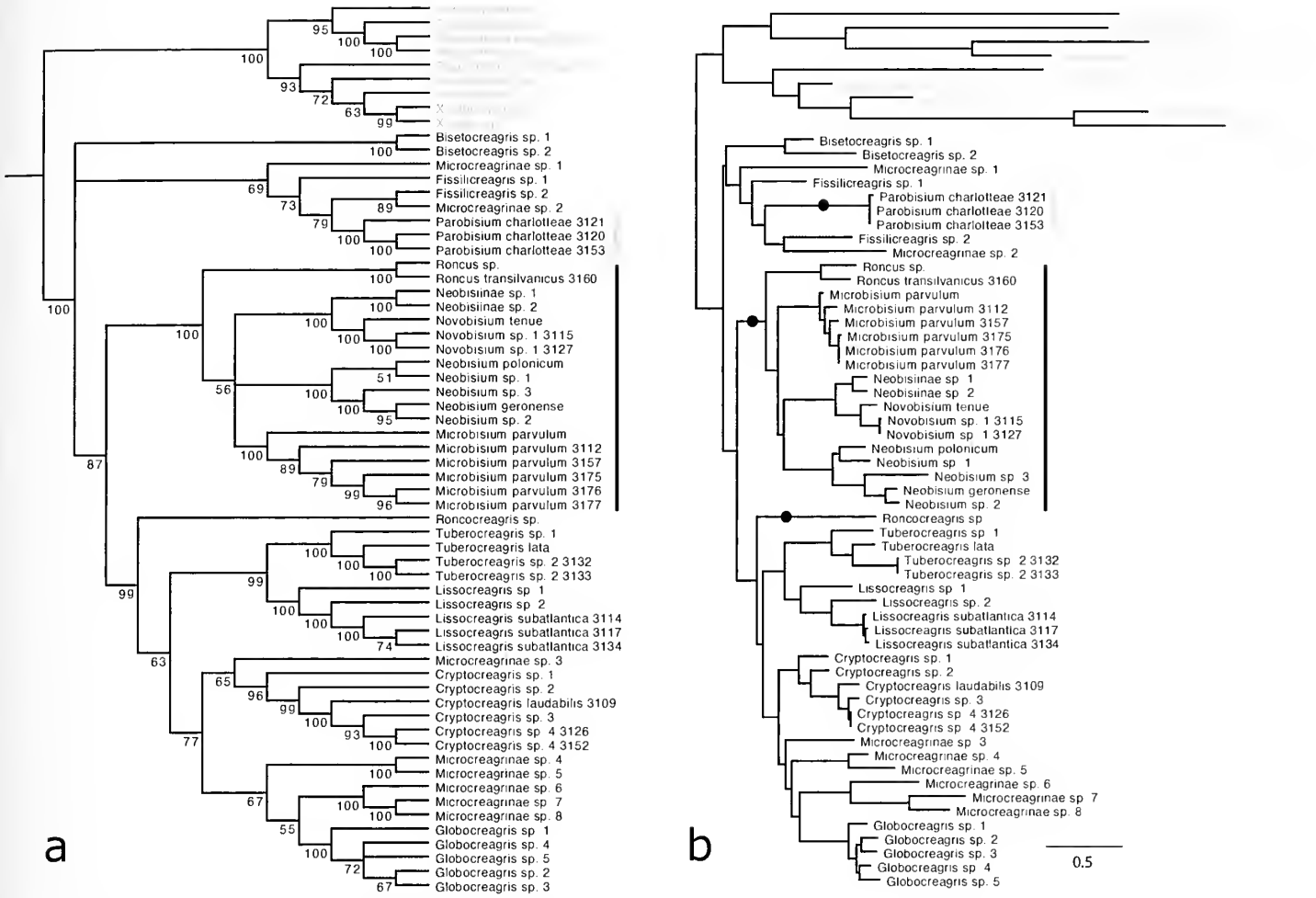

Figure 2.—Maximum likelihood trees of the concatenated dataset. The subfamily Neobisiinae is indicated with a black vertical line to the right of the terminals. *Parobisium charlotteae* is indicated with a light grey vertical line to the right of the terminals. a. Majority-rule consensus tree of 150 maximum likelihood bootstrap replicates. Bootstrap support values are shown below the branches. b. Maximum likelihood tree based on 500 search replicates. Branches are shown proportional to lengths. Black dots indicate the three independent reductions of the galeae within the Neobisiidae.

Neobisiinae was recovered as polyphyletic in our analysis, due solely to the placement of *Parobisium charlotteae* outside of an otherwise well-supported clade containing all other members of this subfamily (Fig. 2). Although we have molecular data for only one of the 16 described *Parobisium* species, striking morphological similarity among all congeners gives us confidence in moving the entire genus out of Neobisiinae. Therefore, we transfer the genus *Parobisium* to the subfamily Microcreagrinae to simultaneously obtain a monophyletic Neobisiinae and resolve character inconsistency for the position of trichobothrium *ist*, which is subdistal in all Neobisiinae taxa included in our study.

Future in-depth molecular and morphological investigations of the Neobisiidae (with more complete taxonomic sampling than in the present study) will be necessary to detect all of the natural subfamily groups. This work should include members of four genera in particular: *Trisetobisium* Ćurčić, 1982; *Occitanobisium* Heurtault, 1977; *Acanthocreagris* Mahnert, 1974; *Insulocreagris* Ćurčić, 1987. *Trisetobisium* and *Occitanobisium* are currently assigned to the Neobisiinae; however, they have a subbasal trichobothrium *ist*. *Trisetobisium* is similar to *Parobisium* in having a reduced galea, and we suspect that it also needs to be removed from the Neobisiinae. *Occitanobisium* is more of a mystery. While members of this genus have reduced galeae, the enlarged trichobothrial areolae and the small size of the chelae makes categorizing the relative position of *ist* subjective. While Harvey & Volschenk (2007) reported that it has a subbasal *ist*, Heurtault (1977) considered *ist* to be subdistal in this genus. *Acanthocreagris* and *Insulocreagris* are currently classified as microcreagrines but they have a subdistal *ist*. *Insulocreagris* also has a reduced galea, which leads us to suspect this genus actually belongs in Neobisiinae. However, *Acanthocreagris* has elongate galeae, leading us to predict that it might not belong to the Neobisiinae, in which case, there could be two lineages with a subdistal *ist*, the Neobisiinae and *Acanthocreagris*.

Results of our molecular phylogenetic analyses indicate that there have been at least three independent reductions of the galea within Neobisiidae (Fig. 2b). These reductions occurred in the most recent common ancestor of the Neobisiinae, the

Table 1.—List of specimens included in this study, indicating locality information for newly sequenced specimens, DNA extraction numbers, body part extracted (wb=whole body, c=chelae), taxonomic identification, and voucher numbers. MCZ voucher numbers are lot numbers, all others are unique specimen numbers. GenBank accession numbers are provided for each sequence. All new COI sequences were amplified with HCO1490 and HCO2198. All new 28S sequences were amplified with LS30F and LS1126R unless otherwise noted by a superscript letter after the GenBank accession number ([a] indicates the sequence was amplified with LS58F and [b] indicates the sequence was amplified with LS1066R).

Taxon/geographic origin	Moore lab DNA Number (body part)	Specimen/lot number	GenBank accession number COI	GenBank accession number 28S
Ideoroncidae				
Ideoroncidae sp. 1		MNHN-JAD70	JN018183	NA
Ideoroncidae sp. 2 USA: AZ	3194 (wb)	UAIC1113067	MF124552	NA
Pseudalbiorix veracruzensis Hoff, 1945	3155 (c)	MCZ 130499	EU559567	MF124381
Xorilbia gracilis	3171 (c)	MCZ 36800	MF124532	MF124395
Xorilbia sp.	3170 (c)	MCZ 36676	NA	MF124394
Syarinidae				
Ideoblothrus bipectinatus Daday, 1897	3179 (c)	MCZ 45816	MF124537	MF124401
Ideoblothrus sp.	3166 (c)	MCZ 130524	MF124390	MF124528
Nannobisium sp.	3167 (c)	MCZ 130525	MF124529	MF124391
Parahyidae				
Parahya submersa Bristowe, 1931		MCZ 130517	EU559548	EU559478
Neobisiidae				
Bisetocreagris sp. 1		MNHN-JAC35	JN018181	JN018395
Bisetocreagris sp. 2		MNHN-JAD69	JN018182	NA
Cryptocreagris landabilis Hoff, 1956 USA: NM	3109 (wb)	UAIC1113005	MF124479	NA
Cryptocreagris sp. 1 USA: CA	3128 (wb)	UAIC1113024	MF124497	MF124358
Cryptocreagris sp. 2 USA: NV	3149 (wb)	UAIC1113045	MF124513	MF124375
Cryptocreagris sp. 3 USA: NM	3135 (wb)	UAIC1113031	MF124504	NA
Cryptocreagris sp. 4 USA: NM	3126 (wb)	UAIC1113022	MF124495	MF124356
Cryptocreagris sp. 4 USA: NM	3152 (wb)	UAIC1113048	MF124516	MF124378
Fissilicreagris sp. 1 USA: CA	3110 (wb)	UAIC1113006	MF124480	MF124341 [b]
Fissilicreagris sp. 2 USA: CA	3129 (wb)	UAIC1113025	MF124498	MF124359 [b]
Globocreagris sp. 1 USA: AZ, Pinaleño Mts.	3108 (wb)	UAIC1113004	MF124478	MF124340
Globocreagris sp. 2 USA: AZ, Santa Catalina Mts.	3104 (wb)	UAIC1113000	MF124474	MF124336
Globocreagris sp. 3 USA: AZ, Santa Catalina Mts.	3106 (wb)	UAIC1113002	MF124476	MF124338
Globocreagris sp. 4 USA: AZ, Rincon Mts.	3105 (wb)	UAIC1113001	MF124475	MF124337
Globocreagris sp. 5 USA: AZ, Santa Rita Mts.	3107 (wb)	UAIC1113003	MF124477	MF124339
Lissocreagris sp. 1		MCZ 130501	EU559555	EU559450
Lissocreagris subatlantica Chamberlin, 1962 USA: TN	3117 (wb)	UAIC1113013	MF124486	MF124347
Lissocreagris subatlantica Chamberlin, 1962 USA: NC	3134 (wb)	UAIC1113030	MF124503	MF124364
Lissocreagris subatlantica Chamberlin, 1962 USA: TN	3114 (wb)	UAIC1113010	MF124484	MF124345
Lissocreagris sp. 2 USA: TN	3124 (wb)	UAIC1113020	MF124493	MF124354
Microbisium parvulum Banks, 1895		MCZ 130502	EU559558	EU559476
Microbisium parvulum Banks, 1895 Mexico	3175 (wb)	MCZ 37879	MF124533	MF124398
Microbisium parvulum Banks, 1895 Mexico	3176 (c)	MCZ 37883	MF124534	MF124399
Microbisium parvulum Banks, 1895 Mexico	3177 (wb)	MCZ 37886	MF124535	MF124400
Microbisium parvulum Banks, 1895 USA: MD	3157 (c)	MCZ 130502	MF124520	MF124383
Microbisium parvulum Banks, 1895 USA: UT	3112 (wb)	UAIC1113008	MF124482	MF124343
Microcreagrinae sp. 1 USA: CA	3130 (wb)	UAIC1113026	MF124499	MF124360
Microcreagrinae sp. 2 USA: CA	3119 (wb)	UAIC1113015	MF124488	MF124349 [b]
Microcreagrinae sp. 3 USA: OR	3123 (wb)	UAIC1113019	MF124492	MF124353
Microcreagrinae sp. 4 USA: CA	3118 (wb)	UAIC1113014	MF124487	MF124348
Microcreagrinae sp. 5 USA: CA	3641 (wb)	UAIC1113114	MF124591	MF124462
Microcreagrinae sp. 6 USA: AZ	3600 (wb)	UAIC1113073	MF124558	MF124421
Microcreagrinae sp. 7 USA: AZ	3604 (wb)	UAIC1113077	MF124562	MF124425
Microcreagrinae sp. 8 USA: AZ	3637 (wb)	UAIC1113110	MF124587	MF124458
Neobisiinae sp. 1	3122 (wb)	UAIC1113018	MF124491	MF124352
Neobisiinae sp. 2	3125 (wb)	UAIC1113021	MF124494	MF124355
Neobisium geronense Beier, 1939		MNHN-JAC22	JN018184	JN018398
Neobisium polonicum Rafalski, 1936		MCZ 130503	EU559556	EU559457
Neobisium sp. 1		MNHN-JAA9	JN018185	JN018399
Neobisium sp. 2		MNHN-JAC14	JN018208	NA
Neobisium sp. 3		MNHN-JAC15	JN018209	NA

Table 1.—Continued.

Taxon/geographic origin	Moore lab DNA Number (body part)	Specimen lot number	GenBank accession number COI	GenBank accession number 28S
Novobisium tenue		MCZ 130504	EU559559	EU559452
Novobisium sp. 1 USA: TN	3127 (wb)	UAIC1113023	MF124496	MF124357
Novobisium sp. 1 USA: TN	3115 (wb)	UAIC1113011	MF124485	MF124346
Parobisium charlotteae Chamberlin, 1962 USA: OR	3120 (wb)	UAIC1113016	MF124489	MF124350
Parobisium charlotteae Chamberlin, 1962 USA: OR	3121 (wb)	UAIC1113017	MF124490	MF124351
Parobisium charlotteae Chamberlin, 1962 USA: OR	3153 (wb)	UAIC1113049	MF124517	MF124379
Roncocreagris sp. Spain	3169 (e)	MCZ 130546	MF124531	MF124393
Roncus sp.		MNHN-JAC28	JN018186	JN018400
Roncus transsilvanicus Beier, 1928 Slovakia	3160 (e)	MCZ 130505	MF124523	MF124385
Tuberocreagris lata Hoff, 1945		MCZ 130508	EU559552	EU559451
Tuberocreagris sp. 1 USA: VA	3131 (wb)	UAIC1113027	MF124500	MF124361
Tuberocreagris sp. 2 USA: VA	3132 (wb)	UAIC1113028	MF124501	MF124362
Tuberocreagris sp. 2 USA: VA	3133 (wb)	UAIC1113029	MF124502	MF124363

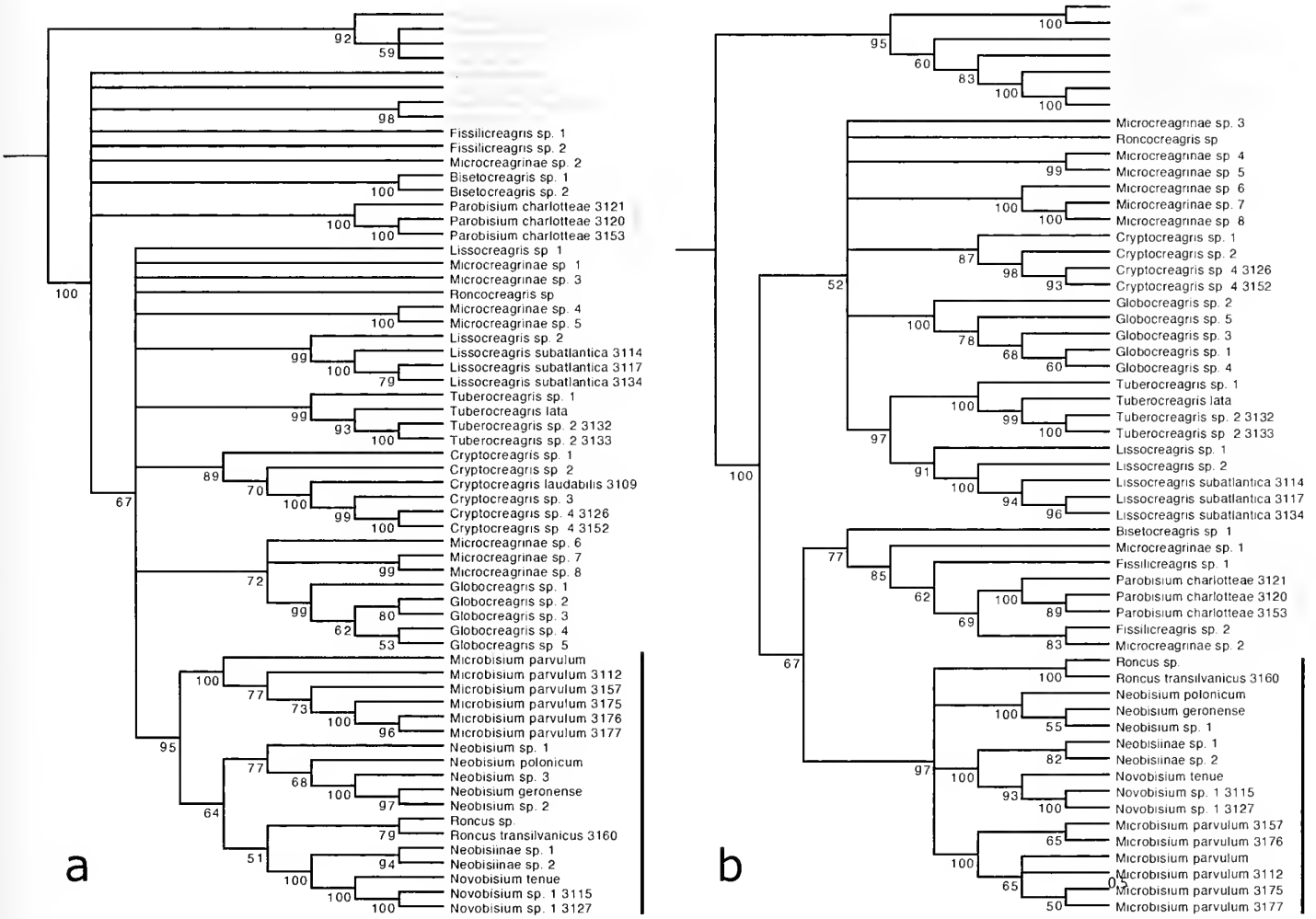

Figure 3.—Majority-rule consensus trees of bootstrap runs based on the single gene matrices. The subfamily Neobisiinae is indicated with a black vertical line to the right of the terminals. *Parobisium charlotteae* indicated with a light grey vertical line to the right of the terminals. a. Consensus tree of 354 maximum likelihood bootstrap replicates for the COI dataset. b. Consensus tree of 252 maximum likelihood bootstrap replicates for the 28S dataset.

Roucocreagris galeonuda species group, and the genus *Parobisium*. We caution future neobisiid researchers that it is sometimes difficult to distinguish between naturally reduced galeae, from those in species that once had elongate galeae but are broken. Cokendolpher & Krejca (2010) used scanning electron microscopy to reveal the evenly rounded microstructure of the galea of *Parobisium*. Indeed, three species previously belonging to *Parobisium* were recently moved to the microcreagrine genus *Bisetocreagris* when it was discovered that the galeae were not reduced but had merely broken off (Mahnert & Li 2016).

Although Kew (1914) stated that the behaviors used to build silken chambers are universally the same across different forms of galea, we suspect that the reduction of the galeae is, in fact, associated with a difference in the way these species spin their chambers as compared with species that have long, branched galeae. It is widely known that in some species adult males have slightly smaller galea with less prominent branches than females (e.g., Kew 1914; Harvey 1995). This sexual dimorphism is particularly associated with species in which only juveniles and females make silken chambers (for molting and brooding respectively). This dimorphism is not present in species in which the both sexes build silken chambers for quiescent periods (aestivation or hibernation). Since such differences in behavior result in minor differences in galea shape between sexes of a single species, we expect there to be a significant behavioral and/or ecological explanation for the drastic reduction of the galea seen in the Neobisiinae, the *Roucocreagris galeonuda* species group, and *Parobisium*. To date, however, such explanations remain elusive. Much work remains to be done on these small and elusive arthropods from ecological, behavioral, and taxonomic perspectives.

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Conspecificity of semaphoronts – the synonymy of *Metadiscocyrtus* with *Propachylus* (Opiliones: Laniatores: Gonyleptidae)

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Abstract. The harvestman genus *Propachylus* Roewer, 1913 is herein revalidated from the synonymy of *Discocyrtus* Holmberg, 1878. Its type species, *Propachylus singularis* Roewer, 1913 has a convoluted taxonomic history, connected to *Discocyrtus fornicatus* Sorensen, 1884 (currently known as *Metadiscocyrtus fornicatus*). The monotypic genus *Metadiscocyrtus* Roewer, 1929 is herein considered a junior subjective synonym of *Propachylus*. *Propachylus singularis* (known only from males) is herein considered a junior subjective synonym of *M. fornicatus* (Sorensen, 1884) (known only from a single female). Accordingly, this species is herein newly combined as *Propachylus fornicatus* (Sorensen, 1884) comb. nov. The males and females of this species are considered congeneric for the first time. As this species does not possess diagnostic characteristics of the concept of Pachylinae *stricto sensu*, it is here removed from Pachylinae but is left unplaced. The geographical distribution of this species is updated to the Brazilian state of Bahia.

Keywords: Arachnida, Grassatores, Neotropics, Pachylinae

The Neotropical harvestman genus *Discocyrtus* Holmberg, 1878 contains ca. 10% of all known diversity in the family Gonyleptidae, and currently has ca. 80 valid species (Kury 2003). It is the largest genus of Pachylinae, which in turn is the largest subfamily of Gonyleptidae. However, previous definitions of Pachylinae have been considered problematic, with the subfamily often regarded as a polyphyletic group (Kury 1994; Pinto-da-Rocha 2002; Hara & Pinto-da-Rocha 2010; Mendes 2011; Caetano & Machado 2013; Pinto-da-Rocha et al. 2014; Carvalho & Kury 2018). It appears that many species of *Discocyrtus* are not especially closely related to the type species (Carvalho & Kury 2018), which is a direct reflection of the artificial generic concepts employed by C. F. Roewer (1913, 1923). This system was followed by Mello-Leitão (1932: 167) and Soares & Soares (1954), who were responsible for the most recent published diagnosis of *Discocyrtus*, where data such as the pattern of armature in the dorsal scutum and the number of tarsomeres ignored a whole range of characteristics that may have phylogenetic value. In recent publications dealing with the genus such inertia was shaken off and this panorama began to change. The revalidation of *Discocyrtanus* Roewer, 1929 by Kury & Carvalho (2016) and the creation of a new Amazonian genus (Carvalho & Kury 2018), led to the removal of seven species from *Discocyrtus* and proposal of a new subfamily based on a morphological phylogenetic analysis.

In this work, we revalidate the genus *Propachylus* Roewer, 1913 and remove it from the synonymy of *Discocyrtus*. *Propachylus* includes a single species that has already been included in three different genera over its history (Sorensen 1884; Roewer 1913; Roewer 1929; Soares & Soares 1954). This species is a large gonyleptid, dark-green colored with red ornamentation, and with unique armature on the dorsal scutum (Fig. 1), which is distributed in coastal regions of the state of Bahia, Brazil.

METHODS

Descriptions of colors use the standard names followed, in parentheses, by the centroid code of the 267 Color Centroids of the NBS/IBCC Color System (Jaffer 2001+) as described by Kury & Orrico (2006). The formula for the tarsomere count, in which the distitarsi of legs I and II are indicated between parentheses, follows Roewer (1935). The formula for the pedipalpal megaspines in which I = large spine and i = small spine is used here following the format established by Kury (1989). The terminology for the scutum outline follows Kury & Medrano (2016). Terminology for chaetotaxy of penis ventral plate follows Kury & Villarreal (2015) for the macrosetae, and Kury (2016) for the microsetae. The term mesotergum (Mello-Leitão 1930) refers to the roughly subrectangular region of the dorsal scutum formed by areas I to IV and circumscribed anteriorly by the carapace, laterally by the lateral margins and posteriorly by the area V (posterior margin of scutum). The diagnoses given here are comparative among four relevant species: the type species of *Amazochroma* Carvalho & Kury, 2018, *A. carvalhoi* (Mello-Leitão, 1941); the type species of *Discocyrtanus* Roewer, 1929, *D. goyazius* Roewer, 1929, both recently removed from *Discocyrtus* (Kury & Carvalho 2016; Carvalho & Kury 2018); the type species of the genus *Discocyrtus*, *D. testudineus* Holmberg, 1878; and the type species of *Mitobates* Sundevall, 1833, *M. triangulus* Sundevall, 1833. *Mitobates* is the type genus of Mitobatinae Simon, 1879, the subfamily closest to *Discocyrtus* according to recent phylogenetic analyses (Pinto-da-Rocha et al. 2014; Carvalho & Kury 2018).

Biogeographical units used here are from the WWF Terrestrial Eco-regions of the World (names starting with "NT"; Olson et al. 2001). They are indicated by colored background areas on the map (Fig. 2).

Scanning electron microscopy was carried out with a JEOL JSM-6390LV at the Center for Scanning Electron Microscopy of Museu Nacional/UFRJ. All measurements are in millimeters (mm).

Abbreviations of the repositories cited are: MCN (Museu de Ciências Naturais. Fundação Zoobotânica, Rio Grande do Sul), MNRJ (Museu Nacional, Universidade Federal do Rio de Janeiro, Rio de Janeiro), MNRJ-HS (Private Collection Helia Soares, presently in MNRJ), SMF (Naturmuseum Senckenberg Sektion Arachnologie, Frankfurt), UFBA (Uni-

Figure 1.—Specimens *in vivo*. *Propachylus fornicatus* (Sorensen, 1884), comb. nov.: A. Male, from Brazil, Bahia, Uruçuca; B. Female, from Brazil, Bahia, Uruçuca. Images courtesy Arthur Anker and Pedro Martins (A, B).

versidade Federal da Bahia) and ZMUC (Zoologisk Museum Universität Kobenhavn, Copenhagen). Other abbreviations used: CL = carapace length, CW = carapace width, AL = abdominal scutum length, AW = abdominal scutum width; VP = ventral plate, macrosetae A1–A4 = basal macrosetae of VP, B = ventro-basal macrosetae of VP, C1–C4 = distal macrosetae of VP, D = dorso-lateral subdistal small setae of VP, E1–E2 = ventro-distal macrosetae of VP (penis).

RESULTS

Discocyrtus fornicatus Sorensen, 1884 was described from a female holotype from "Brazil", without any illustration of this

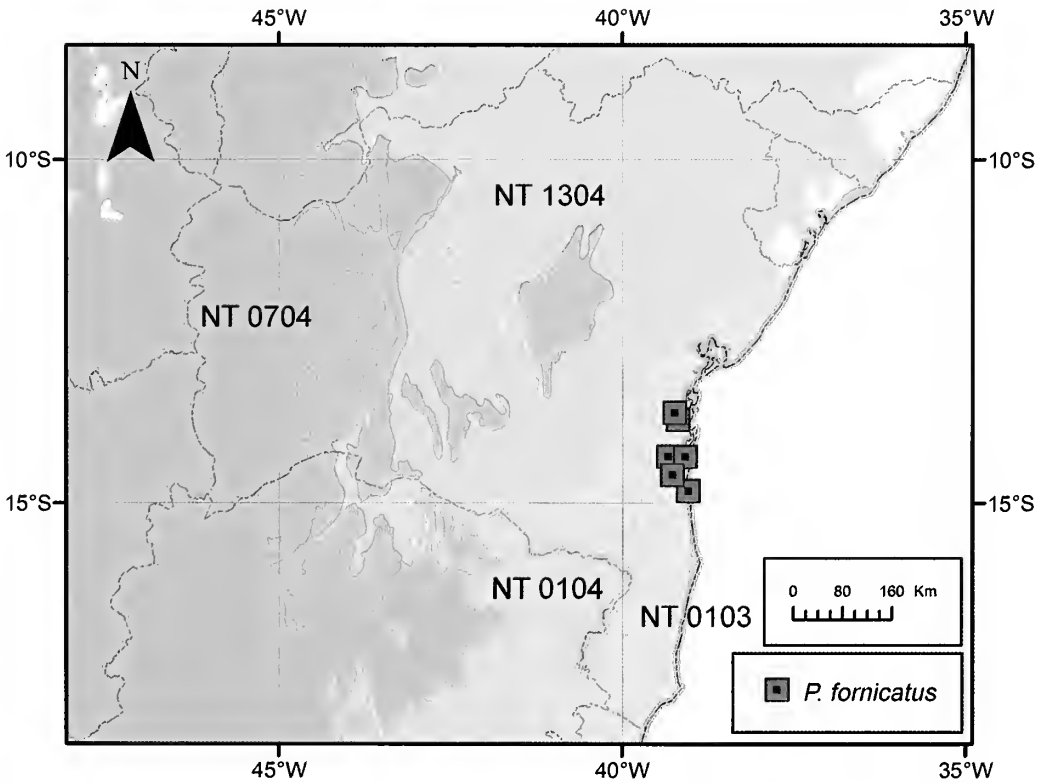

Figure 2.—Bahia state, northeastern Brazil, showing distribution of *Propachylus fornicatus* (Sorensen, 1884), comb. nov. Shaded areas on the background are WWF terrestrial eco-regions: NT 0103 (Bahia coastal forests, in light green), NT 0104 (Bahia interior forests, in dark green), NT 0704 (Cerrado, in orange) and NT 1304 (Caatinga, in yellow).

specimen. The next mention of the name was by Roewer (1913), when he translated the original description from Latin to German without having seen the holotype. He commented that the taxonomic status of *Discocyrtus* was "sehr unsicher" [very uncertain], since only one female specimen was known and it perhaps belonged to another genus (not yet described?), because it differed from the other species in the femur of pedipalps and the pattern of grooves on the abdominal scutum.

In the same publication, Roewer (1913) created the new monotypic genus *Propachylus*, along with the type species *P. singularis* Roewer, 1913, based on a male specimen from "São Paulo", Brazil, although the original holotype label indicates the locality as "Rio de Janeiro", *fide* Acosta (1996). Roewer (1913) provided a drawing of the habitus in dorsal view and a diagnosis which highlighted the pattern formed by the mesotergal grooves and the presence of "thick, erect and large conical spines" in areas I and III.

In his catalogue of Brazilian Laniatores, Mello-Leitão (1923) reported the presence of an unsexed specimen of *D. fornicatus* in his collection collected from "Petrópolis, Rio de Janeiro". There is no evidence that Mello-Leitão had seen the type specimen of this species. Taking into account the original description given by Sorensen, it is possible that this record was based on a misidentification of a female of *D. crenulatus* Roewer, 1913, which is found at Petrópolis (Roewer 1913). This specimen is listed by B. Soares (1945) in his catalog of Opiliones of the MNRJ with no. 1423 (or 776), but it is currently not present in the collection and is probably lost.

Roewer (1929) transferred *D. fornicatus* to a new genus, *Metadiscocyrtus* Roewer, 1929, declaring that after the establishment of further genera such as *Paradiscocyrtus* Mello-Leitão, 1927, *Discocyrtulus* Roewer, 1927, *Discocyrtanus* Roewer 1929, etc., the discrepancies between *D. fornicatus* and the other species of *Discocyrtus* justified a creation of a new genus. He overlooked that the diagnosis of *Metadiscocyrtus* is basically the same as that published sixteen years earlier for *Propachylus*, except for the difference of the shape of the paramedian armature on area III, a common feature of males and females of *Discocyrtus*; e.g., *D. crenulatus* Roewer, 1913 and *D. longicornis* (Mello-Leitão, 1922) (pers. obs.).

Mello-Leitão (1949) described *Propachylus longispinus* Mello-Leitão, 1949 from "Itatiaia", based on male and female syntypes. Based on Roewer's formulae, he placed the species in this genus as he considered the pair of paramedian tubercles of area I to be conspicuous. However, our examination of the drawing published by Mello-Leitão (1949, fig. 6) and the male specimen itself, such a consideration does not make sense. The paramedian tubercles are slightly higher than the adjacent ones, but much smaller than those found in *P. singularis*.

Soares & Soares (1954) published the third fascicle of their monograph of the genera of Neotropical Opiliones, which was dedicated to taxa included at that time in the subfamily Pachylinae. In this work, without any discussion or justification, they transferred both species of *Propachylus* (*P. longispinus* and *P. singularis*) to the genus *Discocyrtus*, where they have since remained. In the identification key of their paper, *Metadiscocyrtus* differs from *Discocyrtus* because it does not have armature on the pedipalpal femur. This is a result of the original description of *D. fornicatus* by Sorensen (1884), whose specimen may have had an intraspecific variation of that character. All the female specimens analyzed here have this part conspicuously armed.

SYSTEMATIC ACCOUNTS

Gonyleptidae Sundevall, 1833
Genus *Propachylus* Roewer, 1913 revalid.

Propachylus Roewer 1913:121; Roewer 1923:440; Mello-Leitão 1923:127; Mello-Leitão 1926:344; Roewer 1929:186; Mello-Leitão 1932:196; Mello-Leitão 1935:100; Muñoz-Cuevas 1973:226 [treated as a junior subjective synonym of *Discocyrtus* Holmberg, 1878, by Soares & Soares (1948)].

Metadiscocyrtus Roewer 1929:258. **Syn. nov.**

Type species.—*Propachylus*: *Propachylus singularis* Roewer, 1913, by monotypy.

Metadiscocyrtus: Discocyrtus fornicatus Sorensen, 1884, by monotypy.

Diagnosis.—Pair of parallel tubercles present on eye mound (pair of divergent spines in *A. carvalhoi*, *D. goyazius*, *D. testudineus* and *M. triangulus*) (Figs. 3A, B, E). Area I higher than others (area III higher than others in *A. carvalhoi*, *D. goyazius*, *D. testudineus* and *M. triangulus*). Paramedian armature of area I formed by a pair of higher and larger tubercles (a pair slightly higher in *A. carvalhoi*, *D. goyazius* and *D. testudineus*, without any highlighted tubercles in *M. triangulus*) (Figs. 3C, E). Anterior outline of area II medially projecting into area I (parallel areas in *A. carvalhoi*, *D. goyazius*, *D. testudineus* and *M. triangulus*) (Figs. 3A, E). Areas I and III with a pair of posterior paramedian extremely higher tubercles (not occurring in other Gonyleptidae). Coxa IV without retrolateral apophysis (same as in *M. triangulus*, present in *A. carvalhoi*, *D. goyazius* and *D. testudineus*) (Fig. 3A). Trochanter IV square-shaped in dorsal view (rectangular-shaped with medial constriction in *A. carvalhoi*, *D. goyazius* and *D. testudineus*, rectangular-shaped in *M. triangulus*) (Fig. 3A). Femur III straight, with approximately the same size of dorsal scutum length (sinuous with approximately the same size of dorsal scutum length in *A. carvalhoi*, *D. goyazius* and *D. testudineus*, straight and extremely longer in *M. triangulus*). Glans sac extended as a dorsal process (without this extension in *A. carvalhoi*, *D. goyazius*, *D. testudineus* and *M. triangulus*) (Fig. 5B). Apex of ventral process of stylus forming an acuminate leaf with smooth flaps (not occurring in another Gonyleptidae – pers. obs.) (Fig. 5D).

Included species.—*Propachylus fornicatus* (Sorensen, 1884) **comb. nov.**

Distribution.—*Propachylus* is only known from the state of Bahia, Brazil.

Propachylus fornicatus (Sørensen, 1884) comb. nov.
(Figs. 1A–B, 3A–I, 4A–E, 5A–D)

Discocyrtus fornicatus Sorensen 1884:633.

Propachylus singularis Roewer 1913:121, fig. 55; Roewer 1923: 441, fig. 554; Mello-Leitão 1923: 127; Mello-Leitão 1932: 196, fig. 111; Acosta 1996: 222. **Syn. nov.**

Metadiscocyrtus fornicatus (Sorensen): Roewer 1929:259; Kury 2003:175.

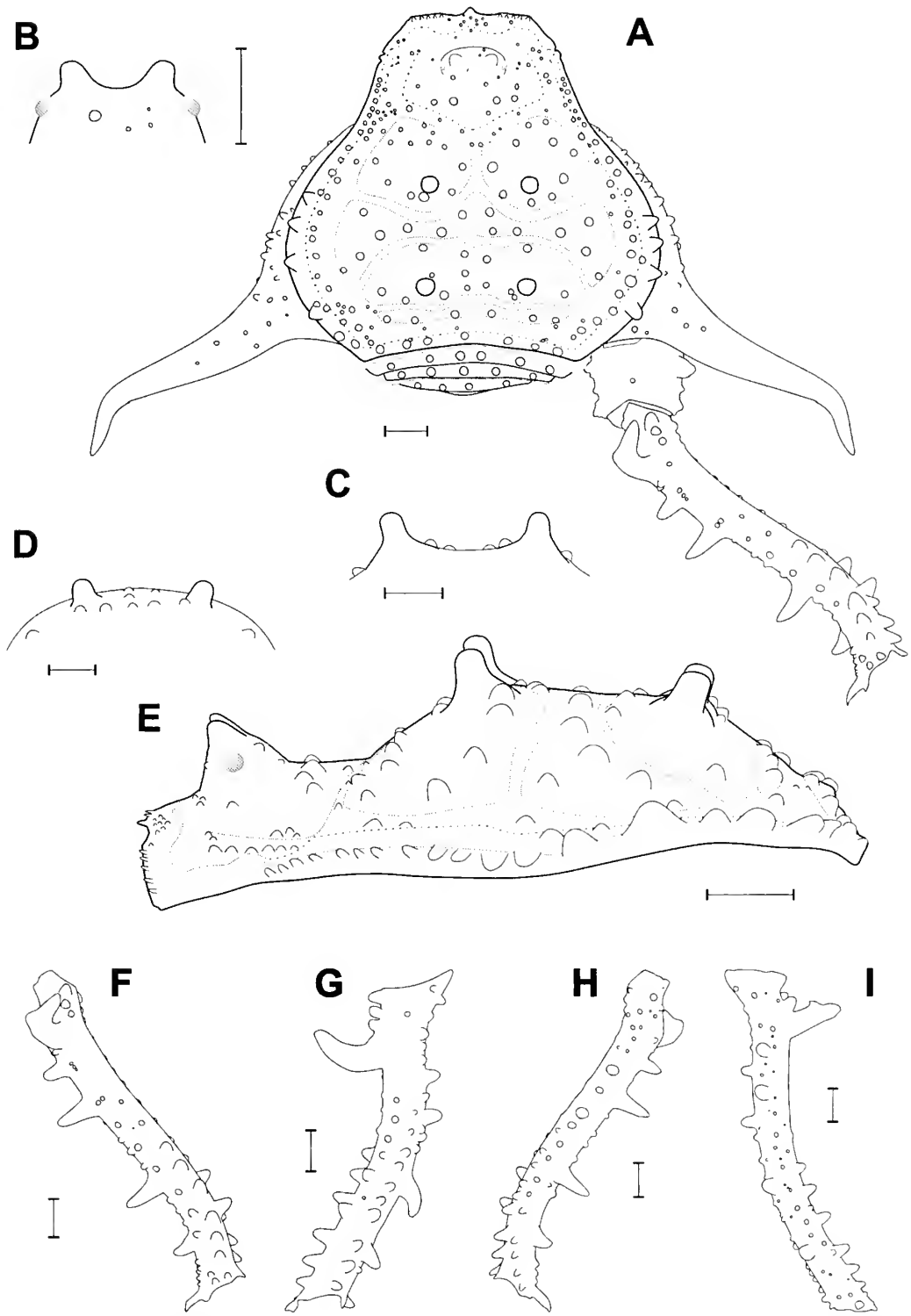

Figure 3.—*Propachylus fornicatus* (Sorensen, 1884), comb. nov., male (MNRJ 8047): A. Habitus, dorsal view; B. Oculariurn, frontal view; C. Armature of scutal area I, anterior view; D. Armature of scutal area III, posterior view; E. Habitus, lateral view; F. Left femur IV, dorsal view; G. Same, prolateral view; H. Same, ventral view; I. Same, retrolateral view. Scale bars = 1 mm.

Discocyrtus singularis (Roewer): Soares & Soares 1954:255; Kury 2003:165.

Type material.—*Discocyrtus fornicatus*: *Holotype female:* Brazil, without further locality data (ZMUC, examined).

Propachylus singularis: *Holotype male:* Brazil, originally labeled "Rio de Janeiro", but reported by Roewer (1913) as "São Paulo", without further locality data; locality probably mistaken (see below) (SMF RI 787, photograph examined).

Published records.—BRAZIL: *Bahia:* (Roewer 1923, as *Propachylus singularis*).

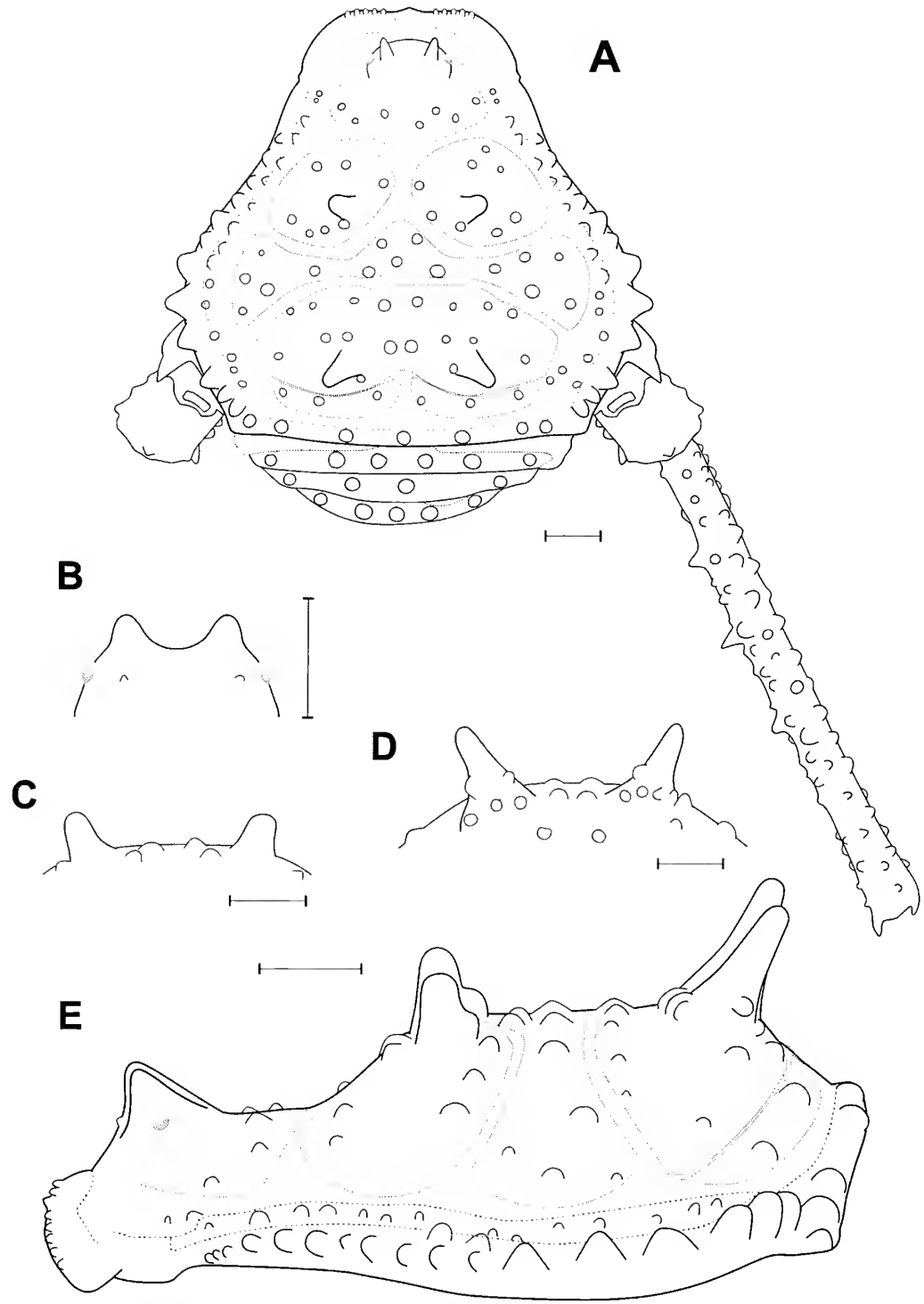

Figure 4.—*Propachylus fornicatus* (Sorensen, 1884), comb. nov., female (MNRJ 8047): A. Habitus, dorsal view; B. Ocularium, frontal view; C. Armature of seutal area I, anterior view; D. Armature of seutal area III, posterior view; E. Habitus, lateral view. Scale bars = 1 mm.

Doubtful records.—BRAZIL: *Petrópolis:* (Mello-Leitão 1923, B. Soares 1945). *Rio de Janeiro:* (Acosta 1996). *São Paulo:* (Roewer 1913). All of these records are *Propachylus singularis*.

Other material examined.—BRAZIL: *Bahia:* Aurelino Leal: 1 ♂ (MNRJ 1942), Fazenda Pedras Pretas, [-14.3166, -39.3272], 21 January 2008, V. Dill; Igrapiúna, Reserva Ecológica da Michelin, [-13.7769, -39.1858]: 1 ♂, 1 ♀ (UFBA 24), 20 January 2009, T. J. Porto; 2 ♂, 1 ♀ (MNRJ 8035), 1 juvenile (MNRJ 8042), 1 ♂, 1 ♀ (MNRJ 8047), 3 ♀ (MNRJ 8052), A.R.S. Andrade; 1 ♀, 1 juvenile (MNRJ 8081), 3 ♂ (MNRJ 8086), 1 ♀ (MNRJ 8142), 1 ♀ (MNRJ 8152), 1 ♂, 1 ♀, 2 juveniles (MNRJ 8262), 2 ♂, 2 ♀ (MNRJ 8273), 24–25 July 2009, A.R.S. Andrade; 1 ♂, 1 juvenile (MNRJ 8057), 1 ♂ (MNRJ 8060), 1 ♂, 2 ♀ (MNRJ 8084), 1 ♂, 1 ♀ (MNRJ 8095), 2 ♂, 2 ♀, 1 juvenile (MNRJ 8098), 3 ♀, 2 juveniles

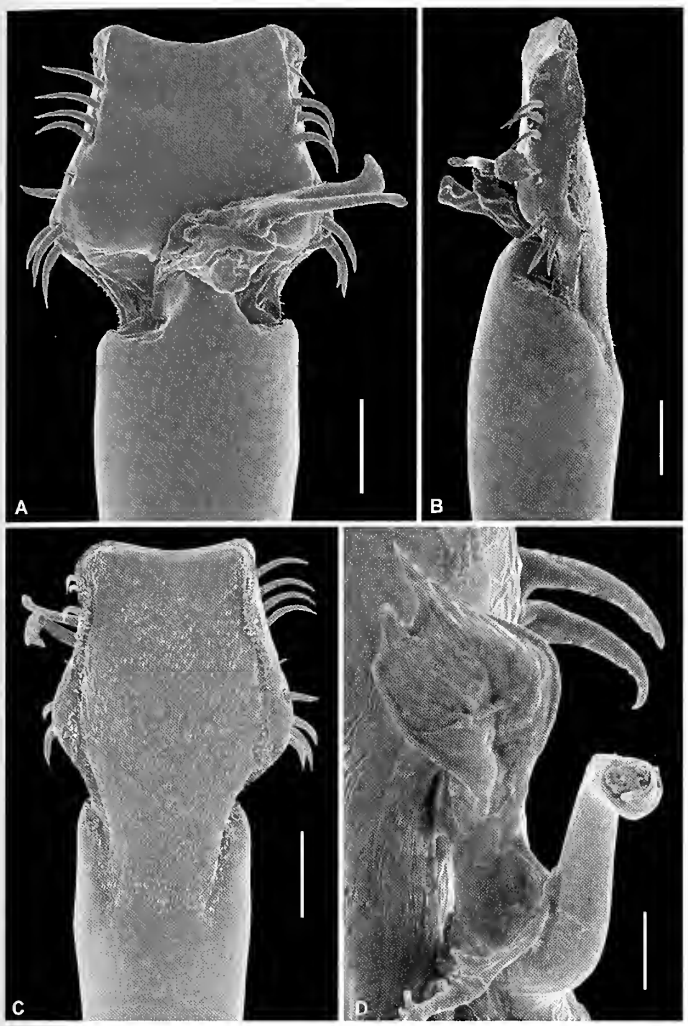

Figure 5.—*Propachylus fornicatus* (Sorensen, 1884), comb. nov., male (MNRJ 8103), genitalia, distal part: A. Dorsal view; B. Lateral view; C. Ventral view. Male (MNRJ 8047): genitalia, distal part: D. Stylus, dorso-lateral view, showing ventral process and flabellum. Scale bars: 100 μm (A–C), 20 μm (D).

(MNRJ 8102), 1 ♀ (MNRJ 8122), 3–6 September 2009, A.R.S. Andrade; 1 ♂, 1 ♀, 4 juveniles (MNRJ 8073), 1 ♀ (MNRJ 8112), 1 ♂, 1 ♀ (MNRJ 8165), 2 ♂, 1 ♀ (MNRJ 8170), 4 ♂, 1 ♀ (MNRJ 8179), 1 ♂, 4 juveniles (MNRJ 8230), 4 ♀, 1 juvenile (MNRJ 8232), 3 ♂, 1 ♀ (MNRJ 8249), 1 ♂ (MNRJ 8256), 29 October –1 November 2009, A.R.S. Andrade; 1 ♂, 1 ♀ (MNRJ 8077), 2 ♂ (MNRJ 8085), 1 ♀ (MNRJ 8131), 1 ♂, 1 ♀ (MNRJ 8149), 1 ♂, 5 ♀ (MNRJ 8157), 2 ♂ (MNRJ 8180), 1 ♀, 1 juvenile (MNRJ 8228), 28–30 January 2010, A.R.S. Andrade; 2 ♂, 3 ♀, 1 juvenile (MNRJ 8033), 5 ♂, 2 ♀ (MNRJ 8048), 1 ♂, 1 ♀, 3 juveniles (MNRJ 8103), 2 ♀ (MNRJ 8134), 1 ♂, 1 juvenile (MNRJ 08168), 4 ♂, 5 juveniles (MNRJ 8171), 2 ♂, 1 ♀ (MNRJ 8176), 4 ♂, 1 ♀, 3 juveniles (MNRJ 8233), 1 ♀ (MNRJ 8237), 3 ♂, 1 juvenile (MNRJ 8252), 27–29 March 2010, A.R.S. Andrade; 5 ♂ (MNRJ 8777), 5–6 October 2011, A. Pérez, B. Huber. Ilhéus, [-14.8167, -39.0333]: 1 ♂ (MNRJ 5431), August 1944; 1 ♂ (MNRJ 9051, Rodovia BR–101, 28 March 2012, V. Dill; 1 ♂, 1 ♀ (MNRJ–HS 0888), Ribeirão do Braço. Itacaré [-14.3224, -39.0793]: 1 ♂ (MCN 1553), 12 January 2003, T.V. Aguzzoli, A. Carvalho; 1 ♂, 1 ♀ (MNRJ 18820), 13–22 April 2006, S.P.S. Alves. Ituberá: 1 ♂ (UFBA 16), Propriedade da Michelin [-13.73, -39.14], 19 May 2007, A. Camacho.

Diagnosis.—As for the genus.

Description, adult male.—*Based on MNRJ 8047*: Measurements: CW 5.4, CL 3.2, AW 9.9, AL 5.5. Leg measurements (Tables 1 and 2), tarsal counts (Table 3).

Dorsum: Dorsal scutum gamma pyriform, widest at area II and highest at area I (Figs. 3A, E). Carapace with few tubercles on posterior region, with two pairs of paramedian contrasting larger tubercles (Fig. 3A). Cheliceral sockets shallow, with a small apophysis in the center (Figs. 3A, E). Ocularium elliptical, medium (ca. 3x the diameter of the eyes), slightly inclined frontwards, placed in the middle of the carapace, armed with a pair of divergent large tubercles inclined frontwards (Figs. 3A, B, E). *Mesotergum* divided into four clearly defined areas. Areas I and IV divided into left and right halves by median groove. Area II anterior lateral border invading space of area I and posterior lateral border invading the space of area III. Area II anterior medial invading space of area I and area IV anterior medial slightly invading space of area III (Fig. 3A). Abdominal scutum lateral borders with two rows of ordinary tubercles from middle of carapace backwards (Fig. 3A). Area I with rounded tubercles forming a frame near the border of this area and a pair of paramedian extremely higher tubercles (same height of the entire eye mound) (Figs. 3A, C, E). Area II anterior with a pair of paramedian rounded tubercles and a pair of lateral rounded tubercles. Area II posterior with a row of 10 rounded tubercles (Figs. 3A, E). Area III with two vertical rows of median rounded tubercles, a pair of rounded tubercles on anterior paramedian border, a pair of posterior paramedian extremely higher tubercles (same height of the entire eye mound) and a row of three rounded tubercles on posterior border (Figs. 3A, D, E). Area IV with a transversal row of three rounded tubercles. Posterior border of dorsal scutum strongly concave. Area V and free tergites with a transversal row of rounded tubercles (Figs. 3A, E).

Venter: Coxa I–III parallel to each other; each with several ventral transverse rows of 9–10 setiferous tubercles (coxa I main row with higher and sharper tubercles). Coxa II retroventral distal with a row of five acuminated tubercles.

Table 1.—Leg measurements of *Propachylus fornicatus* comb. nov., 12 males (major form) examined. Range between larger and smaller values.

	Trochanter	Femur	Patella	Tibia	Metatarsus	Tarsus	Total
Leg I	0.6–0.7	2.8–3.9	1.2–1.5	2.8–3.4	4.3–4.4	2.0 2.3	13.7–16.2
Leg II	1.0–1.2	8.4–10.4	2.0–2.4	7.0–8.8	9.4–11.4	4.1–5.5	31.9–39.7
Leg III	1.2–1.6	7.0–8.4	2.0–2.5	4.0–5.0	7.3–8.7	2.7–3.4	24.2–29.6
Leg IV	1.8–1.9	9.4–11.4	2.3–3.0	6.1–7.6	10.6–13.0	3.2–3.7	33.4–40.6

Table 2.—Leg measurements of *Propachylus fornicatus* comb. nov., 11 females examined. Range between larger and smaller values.

	Trochanter	Femur	Patella	Tibia	Metatarsus	Tarsus	Total
Leg I	0.9–1.0	3.2–3.8	1.4	2.9–3.1	4.3–4.5	1.9–2.0	14.6–15.8
Leg II	1.4	10.0–10.5	1.4–2.3	8.0–8.5	10.7–11.0	5.1–5.2	36.6–38.9
Leg III	1.6–1.7	7.6–8.4	2.2–2.4	4.6–4.9	8.2–8.5	3.2	27.4–29.1
Leg IV	1.5–1.9	8.5–10.9	2.7	6.4–6.7	12.4–13.3	3.2–3.5	34.7–39.0

Coxa III retroventral distal with a row of seven acuminated tubercles. Coxa IV much larger than the others, directed obliquely. Stigmatic area Y-shaped, clearly sunken relative to distal part of coxa IV. Intercoxal bridges well-marked. Stigmata clearly visible. Posterior border of venter with a transversal row of tubercles, increasing in size from the middle to the sides. Free sternites and anal operculum each with one transverse row of setiferous tubercles.

Chelicera: Basichelicerite elongate, bulla well-marked, with marginal setiferous tubercles—one ectal, three posterior, one mesal; hand not swollen.

Pedipalpus: Trochanter with two geminated ventral setiferous tubercles. Femur with a subapical mesal setiferous tubercle and one row of four ventral setiferous tubercles. Patella with dorso-mesal, dorsal and dorso-ectal pair of tubercles. Tibia with two rows of setiferous tubercles: four (IiIi) ventro-mesal and five (IiIii) ventroectal. Tarsus with two rows of setiferous tubercles; three (IIi) ventro-mesal and three (IIi) ventro-ectal.

Legs: Coxa I–III parallel to each other, each with several ventral transverse rows of 9–10 setiferous tubercles (coxa I main row with higher and sharper tubercles). Trochanter I–III each with several dorsal and ventral tubercles. Trochanter I–III ventral with a central highlighted tubercle. Trochanter III proletaral distal with a small apophysis. Femur I–III straight. Femur and tibia I–III with six rows (prodorsal, prolateral, proventral, retroventral, retrolateral and retrodorsal) of small tubercles. Femur II with a little retrodorsal distal spur. Femur III and tibia III with two rows (proventral and retroventral) of acuminated tubercles. Femur III with a developed retrodorsal distal spore. Coxa IV ending distally between area IV and distal border of dorsal scutum (Fig. 3A). Coxa IV with a long prolateral apophysis (forming obtuse angle in relation to coxa) with curvature to posterior on the distal portion (Fig. 3A). Coxa IV covered by tubercles along its entire length. Trochanter IV prodorsal and retrodorsal with apophysis proximal convex prodorsal forming a triangle). Trochanter IV prolateral and retrolateral with a tubercle on medial and distal portion (Fig. 3A). Trochanter IV ventrally covered by tubercles along its entire length. Femur IV sigmoid (Figs. 3A, F–I). Femur IV prodorsal with three large tubercles on proximal, two small tubercles on medial, and six large tubercles on medial–distal portion (Figs. 3A, F–G). Femur IV proventral with a row of 10 large tubercles on medial–distal portion (Figs. 3A, G–H). Femur IV ventral with a row of tubercles along its entire length, where two are larger and higher (on proximal–medial and medial portions, almost forming a cone) (Figs. 3G–I). Femur IV retrolateral with a row of four stout spines (Figs. 3A, F, H–I). Femur IV retrodorsal with a stout hook on proximal portion (curved to prolateral side) followed by a row of small tubercles along its entire length (Figs. 3A, F–G, I). Femur IV retrodorsal ending with a well-developed spur (Figs. 3A, F, H). Patella IV dorsally covered by tubercles. Patella IV proventral and retroventral with row of four and three acuminated tubercles, respectively. Tibia and metatarsus IV with six rows (prodorsal, prolateral, proventral, retroventral, retrolateral and retrodorsal) of acuminated tubercles. Metatarsus IV proventral and retroventral distal with a spur.

Table 3.—Right tarsal (disititarsal) counts of *Propachylus fornicatus* comb. nov., males and females examined.

	♂ ($n = 12$)	♀ ($n = 11$)
Leg I	6(3)	6(3)
Leg II	9–10(3)	5–10(2–3)
Leg III	6–7	4–7
Leg IV	7	7

Penis: VP divided into two regions: distal part inverted trapezoidal with rounded edges, proximal part elliptical (Figs. 5A, C). Ventral surface of VP entirely covered with microsetae of the type 1 (Figs. 5B, C). All macrosetae inserted on lateral of VP: A1–A4, cylindrical, thick, acuminate, with A2–A4 forming inverted triangle on basal third of VP (Figs. 5A–C); B inserted ventrally, proximal to A4 (Figs. 5B, C); C1–C4 slender (circa 70% of size the A), only moderately elongate, forming a tight row on the distal part of VP (Figs. 5A–C); D small, midway between C4 and A1 (Figs. 5A–C); E1–E2 inserted on distal lateral border of VP, E1 between C1 and C2, E2 proximal to C3 (Figs. 5A–C). Glans sac long, arising from middle bulge on podium, extended as a dorsal process (Figs. 5A, B). Stylus cylindrical and slightly S-shaped (Fig. 5D). Apex of stylus without any type of spines or flattening (Figs. 5A–D). Ventral process of stylus cylindrical and distally curved (Figs. 5A, C, D). Apex of ventral process of stylus forming an acuminated leaf with smooth flaps (Figs. 5B–D).

Color (in vivo) (Fig. 1A): Dorsal scutum background Dark Bluish Green (165), with darker shading especially around paramedian tubercles on area I and III. Tubercles of carapace and *mesotergum* Dark Brown (59), main tubercles of eye mound. Paramedian tubercles on area I and III, tubercles on border of dorsal scutum and femur IV main retrodorsal proximal hook Strong Reddish Brown (40). Scutal grooves lighter, Dark Bluish Gray (192). Articular membranes White (263). Chelicerae and pedipalps Dark Yellowish Brown (78) with darker reticle. Legs I to III Dark Brown (59) with darker reticle, and trochanter I–III with distal Light Yellow Green semicircle (119). Leg IV with coxa and trochanter Dark Gray (266) and femur–metatarsus Dark Grayish Purple (229), with tips of apophyses and spines Deep Orange (51).

Color (in alcohol): Dorsal scutum background and tubercles of carapace and *mesotergum* Dark Reddish Brown (44). Main tubercles of eye mound. Paramedian tubercles on area I

and III, tubercles on border of dorsal scutum Moderate Brown (58). Scutal grooves lighter, Strong Brown (55). Articular membranes White (263). Chelicerae and pedipalps Light Olive (106) with darker reticle. Legs I to III Light Grayish Olive (109) with darker reticle and trochanter I–III with semicircle distal Grayish Greenish Yellow (105). Leg IV Grayish Brown (61), with tips of apophyses and spines Light Grayish Brown (60).

Description, adult female.—*Based on MNRJ 8047*: (Figs 1B, 4A–E). CW 4.5, CL 2.5; AW 8.3, AL 5.5. Side of the dorsal scutum edges with lower level of concavity compared to male (Fig. 4A). Ocularium armed with a pair of divergent large tubercles inclined frontwards, a little less divergent when compared to male. Paramedian armature of area I with a posterior callus (Figs. 4A, C, E). Paramedian armature of area III acuminate, turned to posterior (Figs. 4A, D–E). Coxa IV with acuminate prodorsal apophysis (Fig. 4A). Femur IV thinner and less curved when compared to male (Fig. 4A). Fewer and reduced spines on femur IV, only the spore retrodorsal distal (Fig. 4A). No acuminate tubercles on tibia and metatarsus IV.

Minor morphs of males.—*Based on MNRJ 8777:* CW 5.0, CL 3.1; AW 8.9, AL 5.6. Only one minor morph of male was found in all analyzed material; it has: Dorsal scutum sides with lower concavity level between those found in major morphs and without the strongly concave form of posterior border of dorsal scutum; Coxa IV with prolateral apophysis slightly smaller when compared to major morphs; Femur IV thinner, less curved and less armed when compared to major morphs.

Distribution.—*Propachylus fornicatus* has been collected from Bahia at Aurelino Leal, Igrapiúna, Ilhéus, Itacaré, Ituberá and Uruçuca (Fig. 2).

Remarks.—The male holotype of *Propachylus singularis* (SMF RI 787) was reported in the original description from "São Paulo", without further locality data. However, the original label of this specimen has "Rio de Janeiro" as the collecting locality. Both records are highly doubtful. There is a long story of mixed-up or even fictitious records by Roewer (e.g., Helversen & Martens 1972; Pinto-da-Rocha 2002; Kury 2003; Schönhofer 2013). São Paulo and Rio de Janeiro are prominently in terrestrial eco-region NT 0160 (Serra do Mar coastal forests) which has a very different faunal composition than that found in terrestrial eco-region NT 0103 (Bahia coastal forests), where all confirmed records of *P. fornicatus* occur.

DISCUSSION

When Roewer (1913, 1929) proposed the genera *Propachylus* and *Metadiscocyrtus*, he did not have access to the female semaphoront, *Discocyrtus fornicatus*, as he was unable to examine material from ZMUC before 1934, as he later stated (e.g., Roewer 1943, fig. 29; Roewer 1947, fig. 18). He was only able to use the imprecise description provided by Sorensen (1884) to diagnose *D. fornicatus*, who stated: "areae secunda et quarta processibus binis, basi latae impositis, obtusis, instructae" [= areas I and III each with a pair of blunt processes on wide base; translated and adapted to modern terminology by us]. Roewer (1913, fig. 121) in the diagnosis of *Propachylus* stated: "I. und III. Area des Abdominalscutums mit je einem mittleren Paare dicker, aufrechter, großer Kegeldornen bewehrt" [= scutal areas I and III each armed with a paramedian pair of thick, erect, high spines]. Roewer (1929, fig. 258) assumed that the armature of *Metadiscocyrtus* was "1. Area des Scutums mit einem mittleren Tuberkelpaar" [= scutal area I armed with a paramedian pair of tubercles], which contrasted with his (Roewer 1923, fig. 431) diagnosis for *Discocyrtus* "1., 2., 4. u. 5. A, sowie 1.–3. f Tg u. Opa unbewehrt." [= scutal areas I, II, IV and V, as well as free tergites I to III and anal operculum unarmed]. Here we may see the first mistake, as clearly seen in the summary table by Mello-Leitão (1932, fig. 219): the identical armature of area I of males and females of *P. fornicatus* comb. nov. is shown as "$_{00}$" for *Metadiscocyrtus* and "ˆˆ" for *Propachylus*.

Soares & Soares (1954) produced an ambitious synoptic work, full of typographical errors, discrepancies between the key and the diagnoses, and did not evidence new synonymies or combinations. About *Metadiscocyrtus* – on page 231 – they wrote "Área I com dois tubérculos, II com dois espinhos" [= scutal area I with a pair of tubercles, area II with two spines], where they obviously meant area III instead of II (as clearly stated in their diagnosis of *Metadiscocyrtus*). They merged all armature states of area I (unarmed, 2 tubercles, 2 spines) in the diagnosis of *Discocyrtus*, synonymizing *Propachylus*, but not *Metadiscocyrtus*. The single differential character between *Discocyrtus* and *Metadiscocyrtus* is the subapical mesal pedipalpal femur, purportedly present in the former and absent in the latter. However, in another lapse, Soares & Soares (1954) omitted this character in the diagnosis, using it only in couplet 77 of the key. Furthermore, this distinction is fictitious, as shown above.

Now that we have refuted the hypothesis of Roewer (1913) that *Discocyrtus fornicatus* and *Propachylus singularis* represent different species, we can now evaluate (independently of the formal nomenclature) the conflicting hypotheses of Sorensen (1884), which was reaffirmed by Soares & Soares (1954) versus Roewer (1913, 1929) regarding the generic inclusion in *Discocyrtus*.

Propachylus fornicatus evinces a pattern of armature on the dorsal scutum which does not match any of the species of *Discocyrtus sensu* Carvalho & Kury (2018) or even of *Discocyrtus sensu* Soares & Soares (1954), because neither possess well-developed tubercles on area I. In the same way, there are considerable differences in male genitalic features between *P. fornicatus* and core *Discocyrtus*, except for the distribution of macro- and microsetae on ventral plate, e.g., the foliaceus flabellum, the shape of stylus, the outline of ventral plate, and the dorsal process of the glans sac. Therefore, we revalidate the genus *Propachylus* and regard it as distinct from *Discocyrtus*.

The proposal by Sorensen (1884) that *D. fornicatus* should be included in the Pachylinae reflects a very old concept which, after the initial proposal, keeps being repeated by sheer inertia. Modern definitions of Pachylinae (Pinto-da-Rocha et al. 2014; Carvalho & Kury 2018) include a very small core of southern South American species with homogeneous external and genital morphology, which conflicts with that of *P. fornicatus*: absence of microsetae type 1 entirely covering ventral surface of ventral plate; macroseta B very large and inserted laterally; ventral process of stylus as a spiny cone; rectangular ventral

plate; apophyses of coxa IV of male short and directed backwards; and scutal area III without armature.

Among the gonyleptid subfamilies closest to *Discocyrtus* (unranked clade DRMN of Carvalho & Kury 2018), neither the Mitobatinae Simon, 1879 (extreme sexual dimorphism on leg IV; robust pedipalps) nor the Roeweriinae Carvalho & Kury 2018 (T-shaped ventral plate; sigmoid stylus with latero-apical winglets; and macrosetae C elongate and slender) appear as a compelling candidate for the inclusion of *Propachylus*.

Regarding the other nominal subfamilies of Gonyleptidae, none have character states that resemble *Propachylus*, which seems to be very isolated morphologically. In view of the above evidence, *P. fornicatus* is better left unassigned to a subfamily for the time being, while further studies focusing on the phylogenetic relationships of this taxon are conducted.

Furthermore, our study suggests that this genus will remain monotypic, since *D. longispinus* does not have the main characteristics of *Propachylus* and therefore it is provisionally retained in *Discocyrtus* until further phylogenetic work is completed.

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Redescription of the sole species of the enigmatic solifuge genus *Dinorhax* Simon, 1879 (Solifugae: Melanoblossiidae) in Southeast Asia

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Abstract. We present the first recorded description of females of the species *Dinorhax rostrumpsittaci* (Simon, 1877) from solifugae specimens obtained from southern Vietnam. As a result of DNA barcoding using males identified as *D. rostrumpsittaci* and unidentified females, these female specimens made a highly supported cluster with male *D. rostrumpsittaci*. Hereby, we describe the female *D. rostrumpsittaci* and its natural habitats.

Keywords: *Dinorhax rostrumpsittaci*, camel spiders, Indo-china, southern Vietnam, DNA barcoding

While most solifuges are restricted to arid ecosystems (Harvey 2003), *Dinorhax rostrumpsittaci* (Simon, 1877) is unusual for its occurrence in tropical Southeast Asia. In addition to *D. rostrumpsittaci*, a fossil species considered to be closely related to *Dinorhax* has recently been recorded from Cretaceous Myanmar amber (Dunlop et al. 2015; Bartel et al. 2016). *Dinorhax rostrumpsittaci* was originally described by Simon (1877) in the genus *Rhax* Hermann, 1804, possibly on the basis of a single specimen from Gilolo, which is now known as Halmahera, in Indonesia. Simon (1879a) transferred it to the new genus *Dinorhax*, and mentioned southern Vietnam as an additional location. Subsequently, several arachnologists who examined the Solifugae collection in the Muséum National d'Histoire Naturelle (Paris) noted two male specimens from Cochinchina and Bachieu (Kraepelin 1899, 1901, 1908; Roewer 1932, 1933). Cochinchina was a large region in southern Vietnam during the French colonization period, whereas Bachieu [Ba Chieu] is located in Ho Chi Minh City (Vietnam). Roewer (1941) noted that two male specimens from Nha Trang (Vietnam) are deposited at a museum in Leningrad. In addition, Bird et al. (2015) examined a male from Ba Ria-Vung Tau province and another male from Khanh Hoa province; these specimens are currently deposited at the American Museum of Natural History (New York) and Musée Royal de l'Afrique Central (Tervuren), respectively. Although the males of this species are moderately well characterized, females have not been described.

We gathered information about Solifugae from a natural history group in Vietnam via a social networking service and obtained specimens collected by local collaborators (Fig. 1). Among these specimens, we found two females which we confirmed were conspecific to male specimens identified as *D. rostrumpsittaci* using the mitochondrial barcoding gene cytochrome *c* oxidase I. Herein, we describe the female *D. rostrumpsittaci* for the first time and provide additional data on males and their natural habitats.

METHODS

The material examined for the present study is lodged in the Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology, Hanoi (IEBR). The specimens were examined using a Nikon SMZ1270 stereoscope. Images were captured using a Cannon D60 digital camera attached to a Nikon SMZ1270, and focal planes of single image series were combined using Helicon focus 4.2.9. In addition, habitus images were captured using an Olympus TG-4 digital camera.

Molecular analysis.—The specimens used in the present study are shown in Table 1. DNA was extracted from the legs of the specimens using the Chelex-TE extraction protocol (Phung et al. 2016). A fragment of approximately 630 bp of the mitochondrial COI region was amplified using the primer set LCO1490/HCO2198 (Folmer et al. 1994). Each PCR contained 5 μL of 2× PCR buffer (TOYOBO), 2 μL of dNTPs (final concentration, 0.4 mM), 0.3 μL of 10 pmol/μL forward and reverse primers (final concentration, 0.3 μM), 0.2 μL of 1.0 U/μL DNA polymerase KOD FX Neo (TOYOBO KFX-2015), and 0.5 μL of DNA template. The final volume of PCR reaction was adjusted to 10μL by DEPC treated water. The PCR thermal regime comprised one cycle of 2 min at 94°C and 5 cycles of 10 s at 98°C, 30 s at 45°C, and 45 s at 68°C; 35 cycles of 10 s at 98°C, 30 s at 52°C, and 45 s at 68°C; and a final cycle of 7 min at 68°C. The amplified products were incubated at 37°C for 30 min and at 80°C for 20 min with an Illustra™ ExoStar (GE Healthcare, Buckinghamshire, UK) to remove any excess primers and nucleotides. The cycle sequencing reactions were run with an ABI PRISM BigDye Terminator Cycle Sequencing Kit v.3.1 (Applied Biosystems). The sequencing reaction products were purified and concentrated by ethanol precipitation with sodium acetate, and their nucleotide sequences were determined using an automated sequencer (ABI PRISM 3100, Applied Biosystems). The sequences obtained were assembled using ChromasPro 1.7.6 (Technelysium Pty Ltd., Australia). Further, these sequences were aligned using MUSCLE (Edgar 2004) built in MEGA 6.06 (Tamura et al. 2013). The genetic divergence in the K2P model (Kimura 1980) was calculated by the pairwise comparison method. The mitochondrial COI sequences obtained in the present study are deposited in the DNA Data Bank of Japan, and thus will also be available through GenBank.

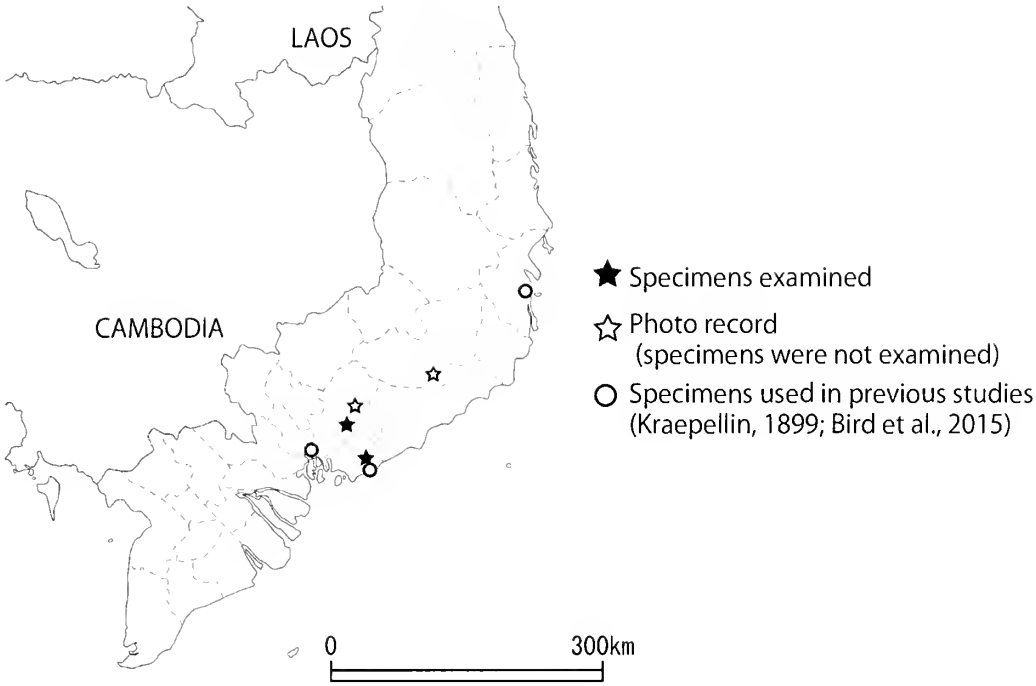


Figure 1.—Distribution of *Dinorhax rostrumpsittaci* in southern Vietnam. Black stars represent the localities of the specimens used in the present study; white stars represent the localities where local collaborators observed specimens (habitat photos shown in Figs. 30–33); and white circles represent the localities of the specimens recorded by Kraepelin (1899) and Bird et al. (2015).

Morphology description.—All measurements are provided in millimeters. Cheliceral terminology, measurements, and dental formulae follow the descriptions by Bird et al. (2015) and Bird & Wharton (2015). Abbreviations used in the present study are as follows: FD, distal tooth on fixed finger; FM, medial tooth on the fixed finger; FP, proximal tooth on the fixed finger; MM, medial tooth on the movable finger; MP, proximal tooth on the movable finger; PF, profondal tooth; RF, retrofondal tooth; MPL, prolateral tooth on the movable finger; MRLC, retrolateral carina on movable finger.

RESULTS

In this study, we obtained approximately 630 bp fragment of the mitochondrial COI fragment from each specimen. The genetic divergence among the specimens is shown in Table 2. Notably, there was no genetic divergence among two unidentified females (SL05, SL06) and two males (SL03, SL04) identified as *D. rostrumpsittaci*, strongly suggesting that the two females are conspecific with *D. rostrumpsittaci*. For our discussion on the high divergence between one male (SL02) and the other specimens, please see Remarks of the species below.

TAXONOMY

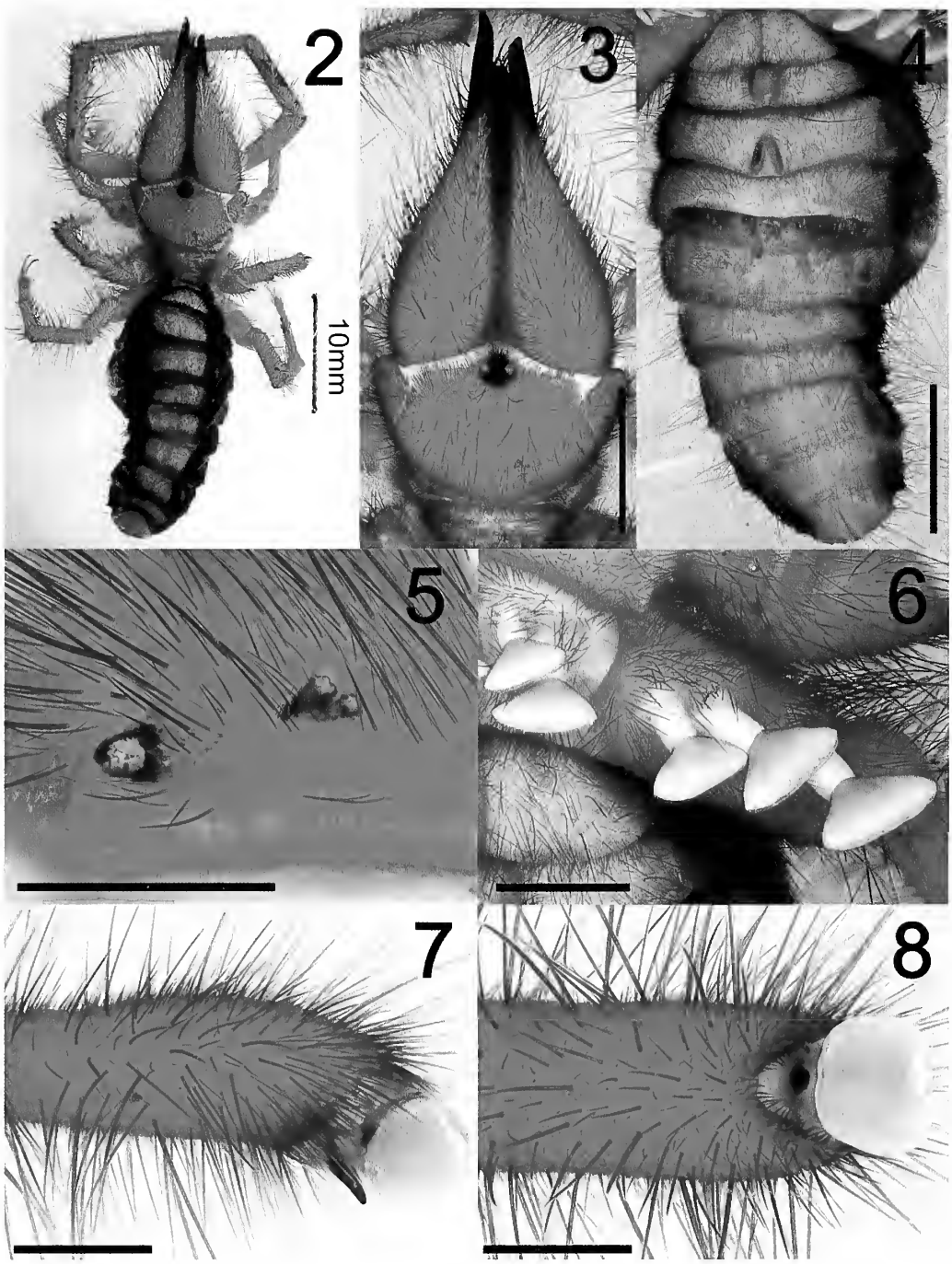
Family Melanoblossiidae Roewer, 1933
Subfamily Dinorhaxinae Roewer, 1933
Genus *Dinorhax* Simon, 1879

Dinorhax Simon 1879a: 125; Simon 1879b: 78; Kraepelin 1901: 41; Roewer 1933: 341.

Remarks.—The sole species of this genus, *D. rostrumpsittaci*, was originally described as a member of the genus *Rhax*,

Table 1.—Specimens used in the present study.

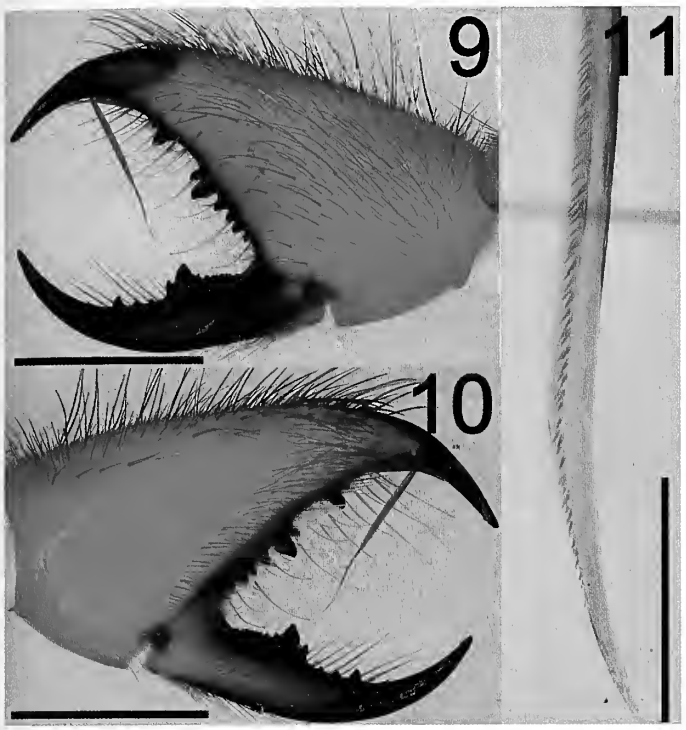
Species	Sex	Code	Locality	Accession No.
D. rostrumpsittaci	Male	SL02	Long Khanh, Dong Nai province	LC328806
	Male	SL03	Xuyen Moc district, Ba Ria Vung Tau province	LC328807
	Male	SL04	Xuyen Moc district, Ba Ria Vung Tau province	LC328808
	Female	SL05	Xuyen Moc district, Ba Ria Vung Tau province	LC328809
	Female	SL06	Xuyen Moc district, Ba Ria Vung Tau province	LC328810



Figures 2–8.—*Duorhax rostrumpsittaci*, male (IEBR_SL03): 2. Habitus, dorsal view. 3. Prosoma, dorsal view. 4. Abdomen, ventral view. 5. Eyespots, lateral view. 6. Malleoli, ventral view. 7. Left pedipalp, prolateral view. 8 Left pedipalp, ventral view. Scale bars = 10 mm (Fig. 2); 5 mm (Figs. 3, 4); 1 mm (Figs. 5, 7, 8); 2 mm (Fig. 6).

Table 2.—Pairwise distances in K2P model are shown below the diagonal. Standard errors are shown above the diagonal.

Specimen	SL02	SL03	SL04	SL05	SL06
SL02		0.009	0.009	0.009	0.009
SL03	0.047		0.000	0.000	0.000
SL04	0.047	0.000		0.000	0.000
SL05	0.047	0.000	0.000		0.000
SL06	0.047	0.000	0.000	0.000	



Figures 9–11.—*Dinorhax rostrumpsittaci*, male (IEBR_SL03): 9. Left chelicera, retrolateral view. 10. Left chelicera, prolateral view. 11. Flagellum. Scale bars = 5 mm (Figs. 9, 10); 1 mm (Fig. 11).

but was transferred to the monotypic genus *Dinorhax* by Simon (1879a) due to the absence of the tarsal claw on tibia I. Currently, this genus is placed in the family Melanoblossiidae (Roewer 1933). However, Bird et al. (2015) suggest that "the inclusion of *Dinorhax* in Melanoblossiidae probably renders this family polyphyletic."

Dinorhax rostrumpsittaci (Simon, 1877)
(Figs. 2–27)

Rhax rostrumpsittaci Simon, 1877: 225 (as *Rhax rostrum-psittaci*).

Dinorhax rostrumpsittaci (Simon): Simon, 1879a: 126, fig. 16 (as *Dinorhax rostrum-psittaci*); Simon, 1879b: 78 (as *Dinorhax rostrum-psittaci*); Kraepelin, 1899: 377 (as *Dinorhax rostrum psittaci*); Kraepelin, 1901: 41, fig. 12 (as *Dinorhax rostrum-psittaci*); Kraepelin, 1908: fig. 56; Roewer, 1932: fig. 142 (as *Dinorhax rostrumpsittaci*); Roewer, 1933: 341, fig. 248 (as *Dinorhax rostrum psittaci*); Roewer, 1941: 123 (as *Dinorhax rostrum psittaci*); Harvey, 2003: 287; Bird et al., 2015: 188, pls. 23d, 25A, 30E, 31C, 41A, 46B, 56A–B, 57A–B.

Material examined.—VIETNAM: *Dong Nai province*: 1 ♂ (IEBR_SL02), Long Khanh district (10°56′41.4″N 107°13′14.9″E), 24 May 2017, Phung Thi Hong Luong leg. (IBER); *Ba Ria Vung Tau province*: 2 ♂ (IEBR_SL03, IEBR_SL04), 2 ♀ (IEBR_SL05, IEBR_SL06), pepper garden, Xuyen Moc district (10°42′21.1″N 107°30′56.7″E), April 2017, Phan Quoc Anh leg. (IBER).

Diagnosis.—*Dinorhax rostrumpsittaci* is distinguishable from other species by having two or three eyespots on each anterolateral propeltidium lobe, a slit-like anus on the venter of terminal segment of abdomen, three dorsal spiniform setae on metatarsus II and III, and one-segmented tarsi II, II, and IV. In males, the cheliceral fixed finger possesses one sessile-form flagellum extending ventrally.

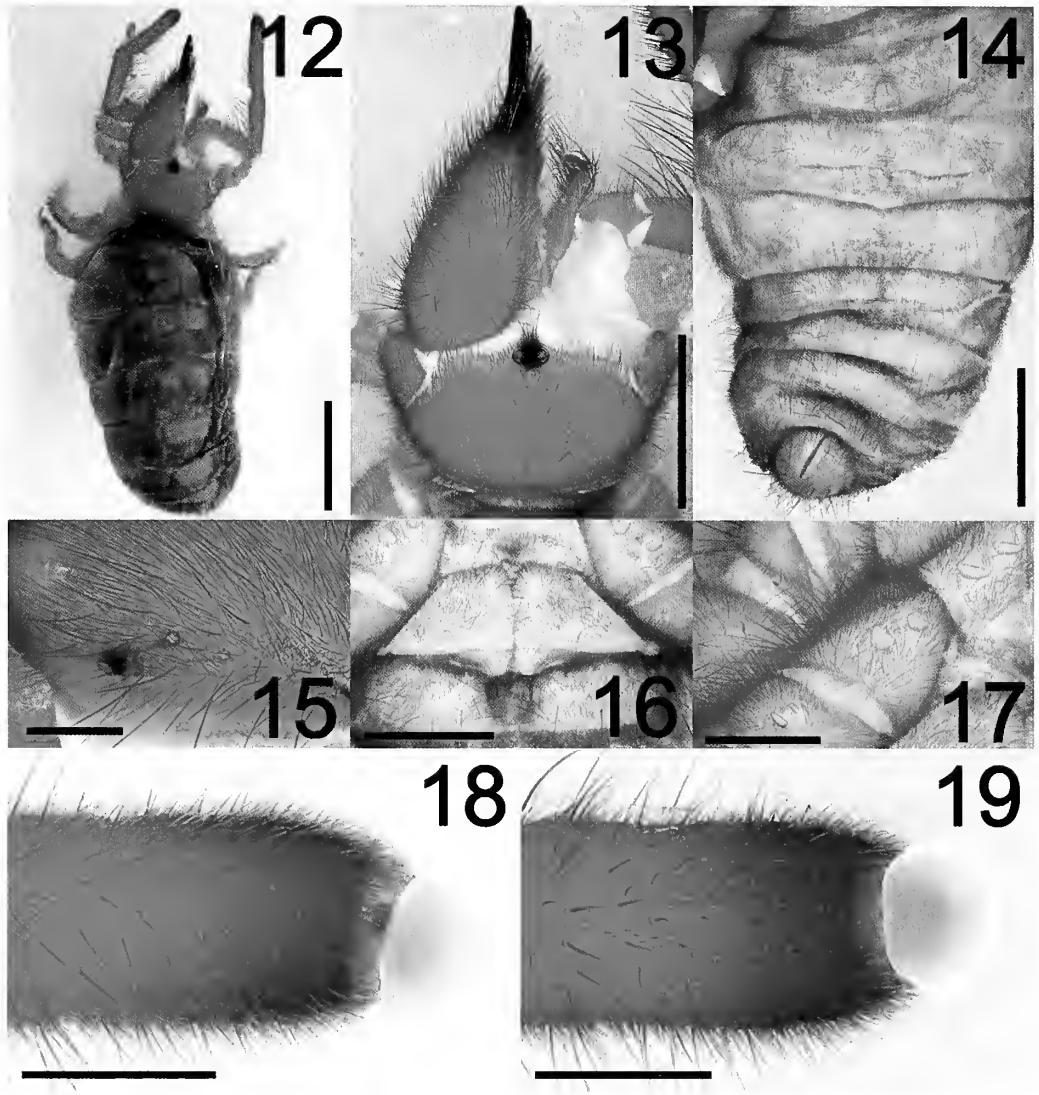
Description.—*Measurements* (3 ♂/2 ♀; means in parentheses): Propeltidium length 60.0–68.2 (63.7)/50.0–55.0 (52.5); width 90.0–112.6 (101.6)/89.0 (89.0). Ocular tubercle length 1.00–1.16 (1.05)/0.73–0.75 (0.74); width 1.36–1.40 (1.38)/1.03–1.13 (1.08). Ocellus diameter 0.50–0.63 (0.59)/0.40–0.43 (0.41). Chelicera length 134.9–153.9 (144.0)/109.5–111.1 (110.3); width 38.0–50.7 (43.1)/35.0–37.0 (36.0); height 62.0–77.7 (69.9)/56.0 (56.0). Pedipalp 260.3–287.2 (272.6)/176.9 (176.9); leg I 195.0–217.1 (206.1)/147.0 (147.0); leg IV 222.2–262.0 (241.5)/164.0–168.0 (166.0).

Male (Figs. 2–11, 24): Propeltidium much wider than long (Figs. 2, 3); median eyes separated by less than one diameter (Fig. 3); two or three small eyespots located near lateral margin of each anterolateral propeltidium lobe (Fig. 5). Abdomen tapering posteriorly, with ventral anus (Fig. 4). Dental formulation of fixed finger: FD-(1)-FM-(1)-FP (3RF or 4RF)(4PF or 5PF); FD minute (Figs. 9, 10, 24). Dental formulation of movable finger: MM-(3)-MP with MRLC; MP accompanying serrated teeth posteriorly (Figs. 9, 10, 24). Flagellum sessile form, directly projecting from basal part of terminal tooth in prolateral view (Fig. 10); shaft bearing longitudinal row of small denticles (Fig. 11). Pedipalp with one apical claw on tarsus (Figs. 7, 8). Leg I without claw. Legs II & III with two apical claws on each tarsus; one dorsal spiniform seta on apex of each postfemur; three dorsal spiniform setae on each metatarsus. Leg IV with two apical claws on tarsus; dorsum without spines; two malleoli on venter of coxa, two on trochanter 1 and one on trochanter 2 (Fig. 6).

Coloration and setation in alcohol preservation (Figs. 2–4). Propeltidium yellowish brown, densely covered with short black setae and sparsely with long black setae. Abdomen densely covered with dark grey setae dorsally, except for cream abdominal sclerites, and ventrally cream. Chelicera yellowish brown, covered with long brownish setae; each terminal tooth of fixed finger and movable finger black, and prolateral margin of fixed finger dentition densely fringed with plumose setae. Pedipalp and legs yellowish brown, covered with brownish setae.

Male variation: Three males were examined for variation. Notably, the number of eyespots and RF in males varies among specimens. Eyespots: Male SL02 has two eyespots on both lateral sides; males SL03 and SL04 have three eyespots on the left side but two on the right side. RF: Males SL02 and SL04 have three RFs, whereas male SL03 has four RFs. The FD is minute among SL02, SL03, and SL04. The sharpness of serration behind MP varies and is relatively blunt in SL02, but it is sharp in SL03 and SL04.

Female (Figs. 12–23, 25–28): Propeltidium almost same as in male (Figs. 12, 13); three small eyespots located at near lateral margin of each anterolateral propeltidium lobe (Fig. 15). Dental formulation of fixed finger: FD-(1)-FM-(1)-FP



Figures 12–19.—*Dinorhax rostrumpsittaci*, female (SL05): 12. Habitus, dorsal view. 13. Prosoma, dorsal view. 14. Abdomen, ventral view. 15. Eyespots, lateral view. 16. Genital operculum, ventral view. 17. Malleoli, ventral view. 18. Pedipalp, prolateral view. 19. Pedipalp, ventral view. Scale bars. 10 mm (Fig. 12); 5 mm (Figs. 13, 14); 1 mm (Fig. 15); 2 mm (Figs. 16, 17); 1 mm (Figs. 18, 19).

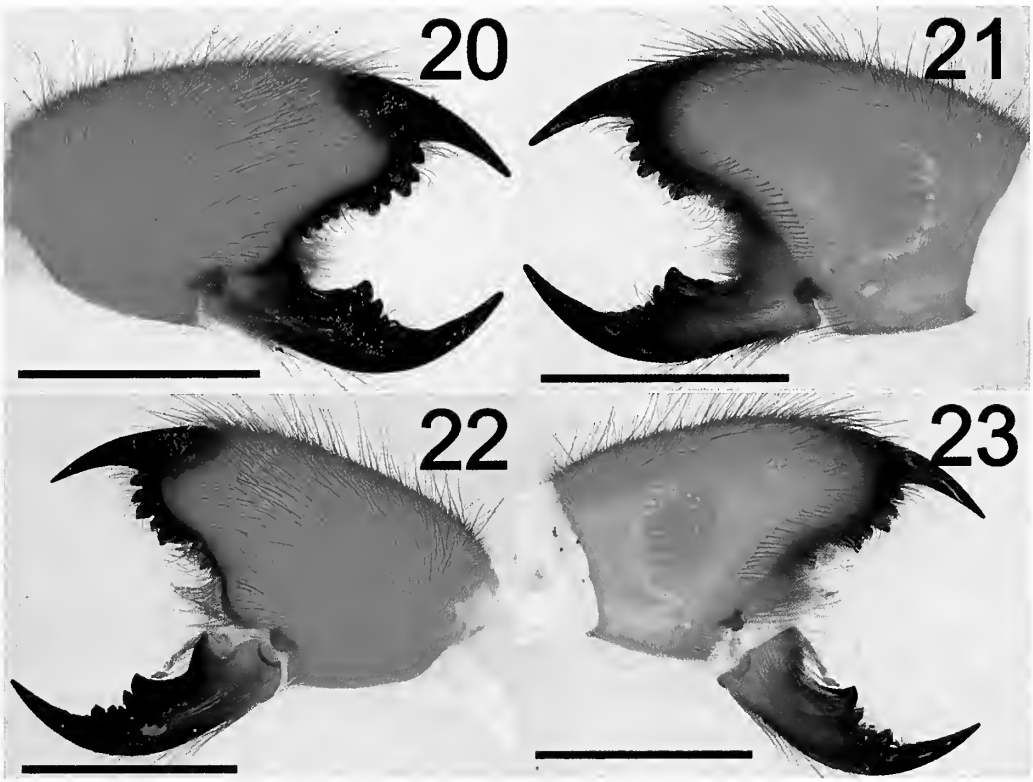
(3RF)(3PF), with one or two small teeth located between RF and PF teeth rows (Figs. 20–23, 25, 27). Dental formulation of movable finger: MM-(3)-MP, with 1 MPL (Figs. 20–23, 26, 28). Abdomen almost as in male (Fig. 14); genital operculum shown in Figure 16. Pedipalp without tarsal claw (Figs. 18, 19). Leg spines and tarsal claws almost same as in male. Malleoli small (Fig. 17).

Coloration and setation almost same as in male (Figs. 12–14).

Female variation: Two females were examined for variation. Female SL05 has one tooth between the RF and PF teeth rows, whereas SL06 has two small teeth. The size of MM occasionally varies between SL05 and SL06 (Figs. 20, 21, 26 vs. Figs. 22, 23, 28).

Distribution.—*Dinorhax rostrumpsittaci* has been recorded from Halmahera, Indonesia (type locality) and Khanh Hoa, Lam Dong, Dong Nai, and Ba Ria-Vung Tau provinces in southern Vietnam. Although Simon (1877) clearly stated the type locality as Iles Moluques: Gilolo [nowadays Moluccas: Halmahera (or Jilolo, Gilolo)] in the original description, all subsequent specimens are from southern Vietnam (Fig. 1), and there are no recorded specimens from Indonesia. Thus, Roewer (1933, 1941) suggested that the record from Halmahera is suspicious, and there is a possibility that the type specimen was somehow mislabeled. However, terrestrial biodiversity inventories in the Malay Archipelago are insufficient to conclude that solifuges do not occur in the Moluccas.

Habitats.—The specimens included in the present study were collected in artificial forest environments such as a pepper garden (Fig. 29). According to the collectors, the specimens were walking on the ground in the daytime after



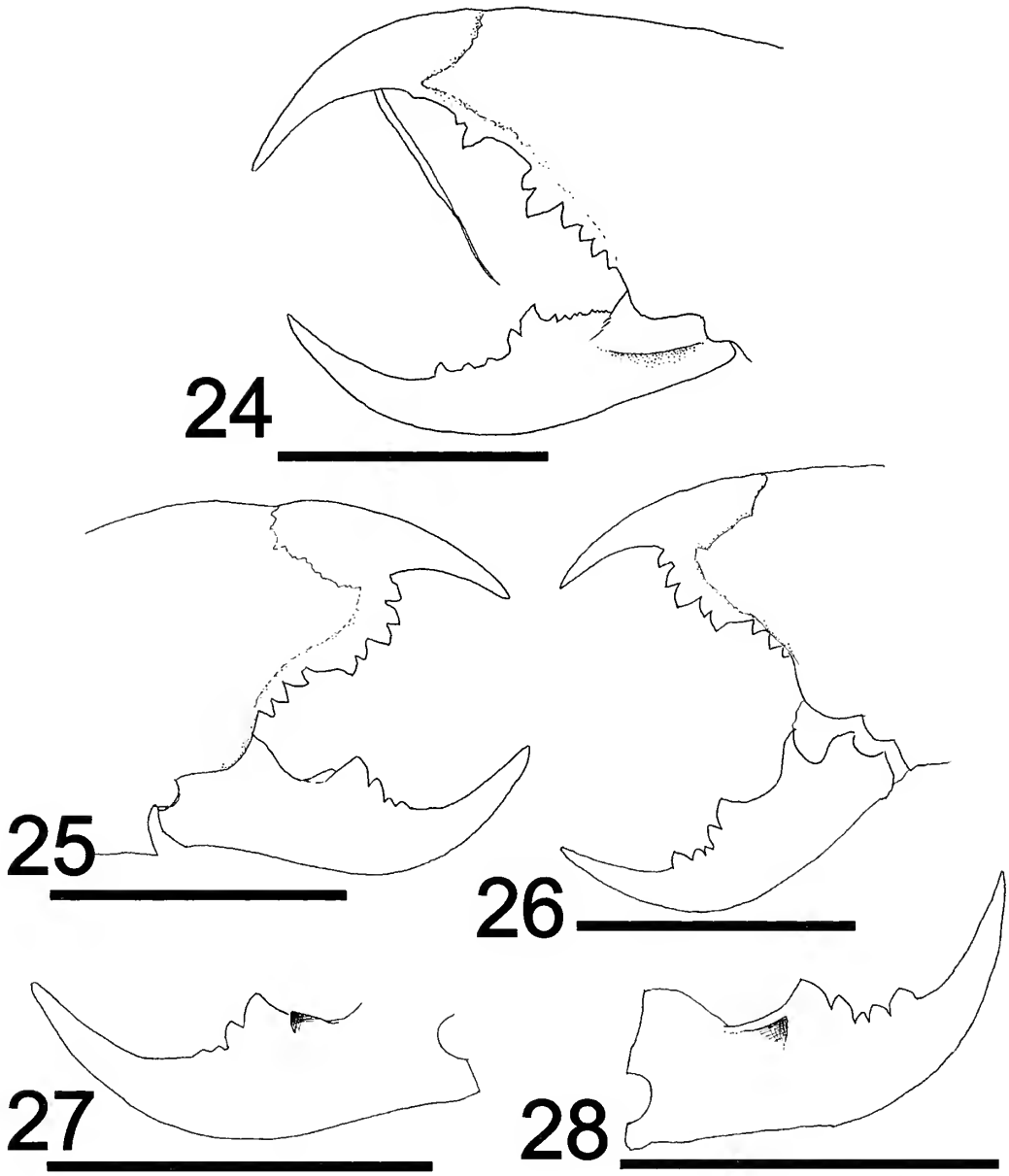
Figures 20–23.—*Dinorhax rostrumpsittaci*, chelicerae, female (SL05, SL06): 20. Right chelicera (SL05), retrolateral view. 21. same (SL05), prolateral view. 22. Left chelicera (SL06), retrolateral view. 23. same (SL06), prolateral view. Scale bars = 5 mm.

rain during the rainy season (May–September in southern Vietnam). In addition, the species has been found in a teak plantation (11°15′01.7″N, 107°24′19.2″E) in Dong Nai province (Figs. 1, 30, 31) and a roadside in a montane area (11°19′05.2″N, 108°05′28.7″E) in Lam Dong province (Figs. 1, 32, 33). These habitats indicate that *D. rostrumpsittaci* may prefer relatively open environments rather than primary forests, although surveys in forest preserves are needed to establish whether this is correct.

Remarks.—The male/female combination was confirmed by DNA barcoding (Table 2), and not unexpected as they were collected from the same location and share various diagnostic features of the genus. Although four specimens (SL03–SL06) collected in the same habitat in Xuyen Moc showed no genetic divergence among them, a male (SL02) specimen collected from Long Khanh has a genetic divergence of 4.70% ± 0.90% from the former specimens in the K2P model (Table 2), without distinct morphological differences. In a study by Maddahi et al. (2016), the average K2P genetic distance varied from 0% to 7.92% within the solifuge species *Galeodes caspius* (Birula, 1890). Therefore, we considered the relatively high divergence value between SL02 and other specimens as merely intraspecific divergence. Although we examined a limited number of specimens, the high mitochondrial divergence between the male from Long Khanh and the others from Xuyen Moc suggests low dispersal ability of *D. rostrumpsittaci*. The fossorial habits and high genetic divergence among the Vietnamese populations of *D. rostrumpsittaci* imply low dispersal ability and it may be possible in the future to use this species to examine the geographical factors affecting the present distribution of this species.

Although *Dinorhax* was placed in the family Melanoblossiidae by Roewer (1933), Wharton (1981) doubted its placement and indicated that the insertion point of the flagellum in *Dinorhax* resembles that in Karschiidae. Bird et al. (2015) noted that *Dinorhax* resembles Rhagodidae and Hexisopodidae in terms of cheliceral morphology, Eremobatidae in terms of the dentition on the chelicera, and the genus *Karschia* Walter, 1889 (Karschiidae) in terms of flagellum morphology. As all other melanoblossiids occur in southern Africa (Harvey 2003), the presence of *Dinorhax* in southeast Asia represents a remarkable disjunct distribution. However, as previously mentioned, the monophyly of Melanoblossiidae has been considered uncertain, and the re-evaluation of the morphological characteristics of *Dinorhax* and further molecular analysis of several gene markers are necessary to conclude the appropriate classification of *Dinorhax*.

Dunlop et al. (2015) draws several hypotheses regarding a potential ancestor of southeast Asian Solifugae on the basis of a Burmese amber specimen, although their discussion relies on an assumption that the amber specimen is closely related to *D. rostrumpsittaci*. Based on the current classification with *D. rostrumpsittaci* as a member of the Melanoblossiidae, some hypotheses by Dunlop et al. (2015) pursue the ancestor of



Figures 24–28.—*Dinorhax rostrumpsittaci*, chelicerae: 24. Left chelicera (SL03), retrolateral view. 25. Right chelicera (SL05), retrolateral view. 26. Left chelicera (SL06), retrolateral view. 27. Movable finger (SL05), right chelicera, prolateral view. 28. Movable finger (SL06), left chelicera, prolateral view. Scale bars = 5 mm.

Figures 29–33.—Habitats of *Dinorhax rostrumpsittaci*. 29. Pepper garden, Xuyen Moe district, Ba Ria Vung Tau province; specimens SL03–06 were collected here. 30. Teak plantation, Dong Nai province (the locality is indicated in Fig. 1 as a white star). A local collaborator observed a *D. rostrumpsittaci* walking across the floor. 31. *D. rostrumpsittaci*, same locality. 32. Di Linh district, Lam Dong province. One of the authors observed a *D. rostrumpsittaci* walking on the side of the road (the locality is indicated in Fig. 1 as a white star). 33. *D. rostrumpsittaci*, same locality. Photo credits: Mr. Phan Quoc Anh (Fig. 29); Mr. Truong Ba Vuong (Figs. 30, 31); and Mr. Quang Duy Hoang (Figs. 32, 33).

African Melanoblossiidae. However, the monophyly of Melanoblossiidae is still controversial considering morphology and molecular phylogeny.

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Molecular and morphological characterization of new species of hypogean *Paradraculoides* (Schizomida: Hubbardiidae) from the arid Pilbara bioregion of Western Australia

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Abstract. The Australian schizomid fauna consists of eight genera distributed across the northern half of the country, and are mostly restricted to rainforest or subterranean ecosystems. Several schizomid species have been previously described from the arid Pilbara bioregion of Western Australia, occurring in subterranean cavities that are accessible only by troglofauna sampling. We document the schizomids from subterranean habitats on the western edge of the Hamersley Range using morphological characters and sequence data from the genes *COI*, ITS2 rRNA, 12S rRNA and 28S rRNA. Several genetic clades were found to cluster geographically, consistent with the geomorphology of the region. Adult males were available for four clades (*Paradraculoides affinis* sp. nov., *P. cochranus* sp. nov., *P. confusus* sp. nov. and *P. trinity* sp. nov.), females only were present for two clades (*P. catho* sp. nov. and *P. obrutus* sp. nov.), and only a single juvenile was available for another clade (*P. celatus* sp. nov.). We hypothesize that each of these clades represent distinct species which are here named and described.

Keywords: Taxonomy, systematics, molecular taxonomy, *Draculoides*

ZooBank LSID: http://zoobank.org:8080/References/11121056-B8A3-486A-9CFA-0C248701D4F4

The hypogean fauna of the arid Pilbara bioregion of northwestern Australia contains numerous radiations of different animal taxa including arachnids (e.g., Harvey & Edward 2007a, b; Harvey & Volschenk 2007; Edward & Harvey 2008; Harvey et al. 2008; Platnick 2008; Burger et al. 2010; Baehr et al. 2012; Alexander et al. 2014), crustaceans (e.g., Poore & Humphreys 1998; Knott & Halse 1999; Karanovic & Marmonier 2003; Wilson 2003; Finston et al. 2004; Finston & Johnson 2004; Karanovic 2006; Guzik et al. 2008), insects and other hexapods (e.g., Smith et al. 2012; Smith & McRae 2014, 2016; Baehr & Main 2016; Trotter et al. 2017), and worms (Brown et al. 2015). Many species have extremely small distributions and most are clearly short-range endemics with challenging conservation issues related to the extraction of iron ore and other minerals (Harvey 2002; Eberhard et al. 2005; Guzik et al. 2011; Harvey et al. 2011).

The small arachnids of the order Schizomida are relatively well-studied in Australia with 53 named species in eight genera occurring in rainforest and subterranean habitats in the northern half of Australia (Harvey 1992, 2000a, b, 2001; Harvey & Humphreys 1995; Harvey et al. 2008; Abrams & Harvey 2015). A recent global phylogeny of Schizomida recovered several exemplar species of the Australian genera *Draculoides* Harvey, 1992, *Julatennius* Harvey, 1992, *Notozomus* Harvey, 1992 and *Paradraculoides* Harvey et al., 2008 among a radiation of the Asian fauna (Clouse et al. 2017), which formed a separate lineage from the American and African fauna.

The Pilbara bioregion of Western Australia currently contains seven described schizomid species in two genera (Harvey et al. 2008; Abrams & Harvey 2015), all of which occur in subterranean voids that are accessible only via specialized troglofauna sampling methods (Halse & Pearson 2014). The identification of schizomid species usually relies on morphological modifications on adult males, especially characters on the flagellum located at the terminal end of the abdomen. Adult females and juveniles are rarely identifiable, especially between closely related species where there are generally no noticeable changes in somatic morphology or in the genital region. Molecular methods have proven extremely useful in species description, delimitation and identification among Pilbara schizomids, with previous descriptions of *Paradraculoides* species providing diagnostic molecular characters for each species based on the mitochondrial barcoding gene *COI* (Harvey et al. 2008). These diagnostic characters were provided to distinguish among seven species of *Draculoides* and *Paradraculoides*.

Recent collecting in an area of the Pilbara from which schizomids have been previously unrecorded (Fig. 1) has revealed several new species of *Paradraculoides* that could be distinguished from each other and from previously described species by differences in the male flagellum. Furthermore, we use a multigene approach to help unravel the relationships and status of the various populations and to describe these species using morphological and molecular approaches.

METHODS

Specimen sampling and morphological examination.—The specimens included in this study were field-sampled using the techniques described by Harvey et al. (2008). The maps were produced with the computer program ArcView version 3.2

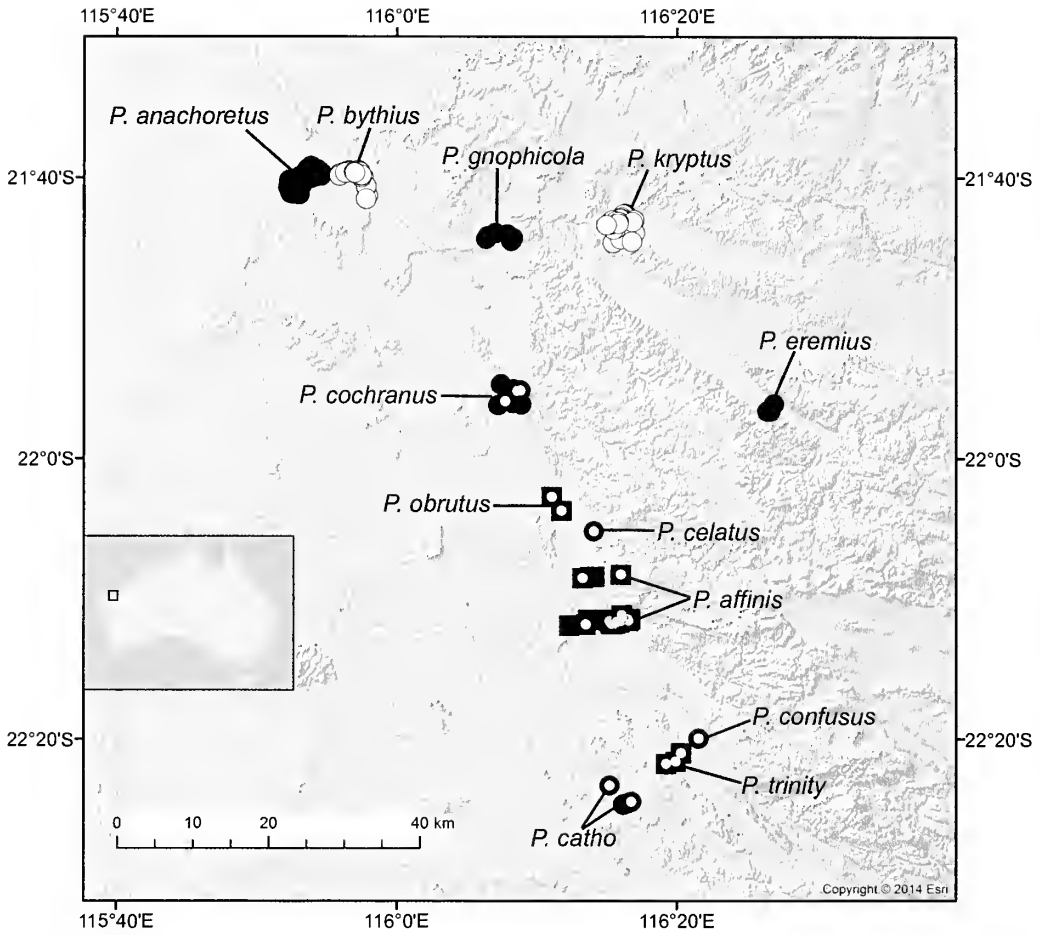

Figure 1.—Map of the western Hamersley Range, Western Australia, showing the distribution of the named species of *Paradraculoides*. The localities of the specimens of the seven new species utilized in the molecular study are depicted with small open circles.

after the relevant locality data were stored in a Microsoft Access database. The descriptions of the new species are presented in alphabetical order, and 'sp. nov.' epithets are removed from the text after the Methods.

All specimens examined in this study are lodged in the Western Australian Museum, Perth (WAM). They were examined with Leica MZ10 and MZ16A dissecting microscopes and a Leica DM2500 compound microscope. The whole-body images were prepared using the Leica Application Suite version 4.6 utilizing multiple images taken with a Leica DFC 500 digital camera, attached to the Leica MZ16A microscope. The female genitalia were examined by dissecting the genital plate from the abdomen and mounting it on a microscope slide in lactic acid.

Terminology of the morphological structures generally follows Harvey (1992) and Reddell & Cokendolpher (1995). The notation of the cheliceral setae follows Manzanilla et al. (2016). The following abbreviations were used for the setae of the flagellum, following Monjaraz-Ruedas et al. (2016): dm1, 4 (dorso-median 1, 4), dl1, 3 (dorso-lateral 1, 3), vm1, 2, 3, 5 (ventro-median 1, 2, 3, 5), vl1, 2 (ventro-lateral 1, 2).

Molecular techniques.—*DNA extraction:* DNA was extracted from legs taken from specimens (Table 1) using a Qiagen DNeasy Blood and Tissue kit, following the manufacturer's protocol, with the exception of the final elution of extracted DNA in 60 µl volume. All specimens except WAM T92518 were sequenced for *COI*, and representative specimens from each *COI* clade were sequenced for the remaining genes.

Amplification: Polymerase Chain Reaction (PCR) was used to amplify two mitochondrial DNA genes (cytochrome *c* oxidase subunit I [*COI*] and 12S rRNA [12S]) and two nuclear rRNA markers (internal transcribed spacer subunit 2 [ITS2] and 28S rRNA [28S]). Amplification of all genes was carried out in 25 µl reactions, which included 1 µl of template DNA, 0.2 µM of each primer, 1 mM dNTPs, 3 mM $MgCl_2$, 1x buffer and 1 unit of taq DNA polymerase (MyTaq) except for *COI* which utilized 2.5 µl DNA template, 0.4 µM of each primer, 0.8 mM dNTPs, 2.5mM $MgCl_2$, 1x buffer and 1.5 units of Taq.

Primers for *COI* differed for different specimens. Some used LCOI490 (5′ – GGTCAACAAATCATAAAGATATTGG – 3′) and HCO2198 (5′ – CTAAACTTCAGGGTGACCAAAAAATCA – 3′) (Folmer et al. 1994), whereas others used schiz-COI-115F (5′ – CAGCCCACGCTTTTGTAATAA – 3′) and schizCOI-863R (5′ – GGCTGCTGTAAAATAAGCTCGT – 3′) (Harvey et al. 2008). Primers for 12S were 12Sai (5′ – AAACTAGGATTAGATACCCTATTAT – 3′) (Kocher et al. 1989) and 12SrOpi (5′ – AAGAGCGACGGGCGATGTGTACAT – 3′) (Sharma & Giribet 2011). Primers for ITS2 were 5.8S2 (5′ – GGGTCGATGAAGAACGCAGC – 3′) and 28SAR (5′ – TCCTCCGCTTATTATATGC – 3′) (Rix et al. 2010). Primers for 28S were

Table 1.—Specimens used in the moleecular analysis, with GenBank accession numbers. All locations are situated in Western Australia.

Species	WAM registration number	Biota code	Speeimens	Locality	Coordinates	*COI*	ITS2	28S	12S
Draculoides bramstokeri Harvey & Humphreys, 1995	T122887		1 juvenile	Barrow Island	20°49′12″S, 115°26′04″E	KY573150	MG913092	KY573737	MG912998
Paradraculoides anachoretus Harvey, et al. 2008	T127081		1 juvenile	Mesa A, 43.23 km W. of Pannawoniea	21°39′33″S, 115°54′27″E	MG913036	MG913102	MG913023	MG913010
Paradraculoides bythius Harvey, et al. 2008	T127091		1 juvenile	Mesa B, 38.26 km W. of Pannawoniea	21°39′36″S, 115°57′20″E	MG913037	MG913104	MG913022	MG913009
Paradraculoides erentius Abrams & Harvey, 2015	T114968		Holotype ♂	Bungaroo, 35.4 km SE. of Pannawonica	21°56′37″S, 116°26′26″E	KU291135	MG913100	MG913024	MG913011
Paradraculoides gnophicola Harvey, et al. 2008	T116008		1 ♀	Mesa G, 22.4 km SW. of Pannawoniea	21°44′03″S, 116°07′52″E	MG913035	MG913103	MG913021	MG913007
Paradraculoides kryptus Harvey, et al. 2008	T120146		1 juvenile	Mesa K, 10 km SW. of Pannawoniea	21°42′49″S, 116°16′03″E	ASN79104	MG913101	MG913020	MG913008
Paradraculoides affinis sp. nov.	T54122	UCRC076T1-1	1 ♀	Upper Cane	22°11′35.58″S, 116°15′54.89″E	MG913067	MG913109	-	-
	T54125	UCRC064T2-2	1 juvenile	Upper Cane	22°11′46.38″S, 116°15′21.23″E	MG913048	-	-	-
	T54130	UCRC029T3-1	2 ♀	Upper Cane	22°11′38.66″S, 116°15′52.71″E	MG913044	MG913110	-	-
	T54131	UCRC021T3-1	1 juvenile	Upper Cane	22°11′35.31″S, 116°15′10.72″E	MG913043	MG913111	-	-
	T54132	UCRC069P2T3-1	2 juveniles	Upper Cane	22°11′44.04″S, 116°15′35.26″E	MG913045	MG913112	-	-
	T54133	UCRC029P2T1-1	1 juvenile	Upper Cane	22°11′38.66″S, 116°15′52.71″E	MG913041	-	-	-
	T54135	UCRC040P2T1-1	1 juvenile	Upper Cane	22°11′32.16″S, 116°16′31.27″E	MG913046	-	-	-
	T54137	UCRC053P2T2-1	1 juvenile	Upper Cane	22°11′31.99″S, 116°16′06.82″E	MG913056	-	-	-
	T54138	UCRC076T2-1	1 ♀	Upper Cane	22°11′35.58″S, 116°15′54.89″E	MG913057	MG913113	-	-
	T54140	UCRC076P2T2-2	1 juvenile	Upper Cane	22°11′35.58″S, 116°15′54.89″E	MG913058	-	-	-
	T54143	UCRC051P2T3-1	1 juvenile	Upper Cane	22°11′24.48″S, 116°16′06.75″E	MG913059	MG913114	-	-
	T54144	CBRC208P2T-1	1 juvenile	Cardo	22°11′48.36″S, 116°13′28.01″E	MG913049	MG913115	-	-
	T54146	UCRC051P2T2-1	1 juvenile	Upper Cane	22°11′24.48″S, 116°16′06.75″E	MG913060	MG913116	-	-
	T54147	UCRC062P2T3-1	1 juvenile	Upper Cane	22°11′42.91″S, 116°15′16.30″E	MG913072	-	-	-
	T92518	UCRC040P3T1-1	1 ♀	Upper Cane	22°11′33″S, 116°16′31″E	-	MG913117	-	-
	T92519	UCRC078P3T3-1	1 ♀	Upper Cane	22°11′33″S, 116°15′49″E	MG913061	-	-	-
	T92520	UCRC051P3T2-2	1 ♀	Upper Cane	22°11′25″S, 116°16′07″E	MG913068	-	-	-
	T92523	UCRC051P3T1-3	1 ♂	Upper Cane	22°11′25″S, 116°16′07″E	MG913040	-	-	-
	T92524	UCRC069P3T1-1	1 ♀	Upper Cane	22°11′44″S, 116°15′35″E	MG913047	MG913118	-	-
	T92525	UCRC042P3T2-1	1 ♀	Upper Cane	22°11′27″S, 116°16′28″E	MG913062	-	-	-
	T92529	UCRC062P3T3-1	1 ♀, 3 juveniles	Upper Cane	22°11′43″S, 116°15′16″E	MG913042	MG913108	-	-
	T92533	CBRC08P3T3-1	1 ♀, 1 juvenile	Cardo	22°08′15″S, 116°15′59″E	MG913076	-	-	-
	T93143	UCRC069P3T2-1	1 ♀	Upper Cane	22°11′44″S, 116°15′35″E	MG913050	-	-	-
	T93202	UCRC021P4T2-1	3 ♀	Upper Cane	22°11′35″S, 116°15′11″E	MG913063	-	-	-

Table 1.—Continued.

Species	WAM registration number	Biota code	Specimens	Locality	Coordinates	*COI*	ITS2	28S	12S
	T93211	UCRC040P5T2-3	Holotype ♂	Upper Cane	22°11′32″S, 116°16′31″E	MG913053	MG913119	MG913026	-
	T93212	UCRC040P5T3-3	1 ♂, 1 ♀, 1 juvenile	Upper Cane	22°11′32″S, 116°16′31″E	MG913071	MG913120	-	-
	T93215	UCRC076P5T3-3	1 ♀	Upper Cane	22°11′36″S, 116°15′55″E	MG913069	-	-	-
	T93216	UCRC029P5T3-2	1 ♀, 1 juvenile	Upper Cane	22°11′39″S, 116°15′33″E	MG913070	-	-	-
	T93221	UCRC053P5T3-2	1 ♀	Upper Cane	22°11′32″S, 116°16′07″E	MG913064	MG913121	-	-
	T98313	UCRC040p7T2-1	1 ♂, 2 juveniles	Upper Cane	22°11′32″S, 116°16′31″E	MG913051	-	MG913031	MG913006
	T98316	UCRC040P7T3-1	1 ♀	Upper Cane	22°11′32″S, 116°16′31″E	MG913054	-	MG913028	-
	T98317	UCRC040P7t1-3	1 ♀	Upper Cane	22°11′32″S, 116°16′31″E	MG913055	-	MG913027	MG913003
	T98318	UCRC076P7T1-1	1 ♀	Upper Cane	22°11′36″S, 116°13′55″E	MG913065	-	-	-
	T98319	UCRC093P7T2-1	2 juveniles	Upper Cane	22°11′19″S, 116°16′03″E	MG913066	-	-	-
	T98321	CBRC192P7T1-1	2 ♀	Cardo	22°08′31″S, 116°13′15″E	MG913077	-	-	MG913000
	T98701	PH16P8T1-1	1 juvenile	Upper Cane	22°11′09″S, 116°16′03″E	MG913074	-	MG913025	MG913001
	T98702	UCRC040P8T3-5	1 ♀	Upper Cane	22°11′32″S, 116°16′31″E	MG913052	-	-	MG913002
	T98705	PH16P8T2-1	1 juvenile	Upper Cane	22°11′09″S, 116°16′03″E	MG913075	-	MG913034	-
Paradraculoides catho sp. nov.	T93192	CWRC281P4T2-1	Holotype ♀	Catho	22°23′18″S, 116°15′11″E	MG913078	MG913125	MG913019	-
	T98700	CWRC166P8T2-1	1 juvenile	Catho	22°24′28″S, 116°16′44″E	MG913079	-	MG913018	MG912999
Paradraculoides celatus sp. nov.	T98698	KBRC039P8T2-5	Holotype juvenile	Kens Bore	22°05′11″S, 116°14′03″E	MG913085	MG913105	MG913033	MG913012
Paradraculoides cochranus sp. nov.	T92538	RNRC083P3T2-4	1 juvenile	Jewell & Cochrane	21°55′55″S, 116°07′43″E	MG913089	MG913097	-	-
	T93191	RNRC184P4T2-3	1 juvenile	Jewell & Cochrane	21°55′09″S, 116°08′49″E	MG913086	MG913094	-	-
	T93197	RNRC083P4T1-3	Paratype ♀, 1 juvenile	Jewell & Cochrane	21°55′55″S, 116°07′43″E	MG913090	MG913098	-	-
	T93229	RNRC083P5T1-3	Holotype ♂	Jewell & Cochrane	21°55′55″S, 116°07′43″E	MG913091	MG913099	-	-
	T93231	RNRC189P5T1-1	1 ♀	Jewell & Cochrane	21°55′11″S, 116°08′35″E	MG913088	MG913093	-	-
	T93232	RNRC184P5T3-1	1 ♂	Jewell & Cochrane	21°55′09″S, 116°08′49″E	MG913087	MG913096	-	-
Paradraculoides confusus sp. nov.	T93142	TBRC014T1	Holotype ♂	Trinity	22°19′57″S, 116°21′32″E	MG913080	MG913122	MG913017	-
Paradraculoides obrutus sp. nov.	T54175	KBRC023P2T2-1	Holotype ♀	Kens Bore	22°03′42.7″S, 116°11′44.29″E	MG913038	MG913106	MG913015	-
	T98320	KBRC096P7T2-1	Paratype ♀	Kens Bore	22°02′43″S, 116°11′03″E	MG913039	MG913107	MG913014	-
Paradraculoides trinity sp. nov.	T92510	TBRC036P3T3-4	Holotype ♂	Trinity	22°21′45″S, 116°19′13″E	MG913083	MG913123	MG913016	-
	T92511	TBRC023P3T3-1	Paratype ♂	Trinity	22°21′35″S, 116°19′54″E	MG913082	MG913124	-	-
	T92515	TBRC023P3T1-1	Paratype ♀	Trinity	22°21′35″S, 116°19′54″E	MG913084	-	-	-
	T98704	CWRC166P8T2-1	1 juvenile	Trinity	22°20′59″S, 116°20′17″E	MG913081	-	-	-

28S_D1F (5′ – GGGACTACCCCCTGAATTTAAGCAT – 3′) and 28Sb (5′ – TCGGAAGGAACCAGCTACTA– 3′) (Nunn et al. 1996; Park & O'Foighil 2000).

Reactions for *COI* based on LCOI490 and HCO2198 were run on a 'touch-up' program: 95°C for 5 min; then seven cycles of 95°C for 30 s, 40°C for 30 s, and 72°C for 45 s; then 35 cycles of 95°C for 30 s, 50°C for 30 s, and 72°C for 45 s; with a final extension at 72°C for 10 min. *COI* reactions based on schiz-COI-115F and schiz-COI-863R were run on 94°C for 2 min; then 35 cycles of 94°C for 30 s, 48°C for 20 s, and 72°C for 15 s; with a final extension at 72°C for 2 min. Reactions for 12S, ITS2 and 28S were run at 95°C for 5 min; then 35 cycles of 95°C for 30 s, the annealing temperature for 30 s, and 72°C for 60 s; with a final extension at 72°C for 10 min. The annealing temperatures for 12S, ITS2 and 28S were 44°C, 50°C and 55°C, respectively. PCR products were visualized on E-Gel® pre-cast agarose gels (Life Technologies, Melbourne, Australia). Bi-directional sequencing was carried out at the Australian Genome Research Facility (AGRF), and for newly prepared specimens, sequences and workflows were managed in the Geneious software package (version 7.1.5) (Kearse et al., 2012), using the LIMS Biocode plug-in (online at http://www.mooreabiocode.org).

Sequence editing and phylogenetic analysis: For phylogenetic analyses, the other five described *Paradraculoides* species were included, and the tree was rooted on *Draculoides bramstokeri* Harvey & Humphreys, 1995. This species was selected as the outgroup based on a multi-gene dataset showing that it is sister to most of the other species of *Draculoides* and *Paradraculoides* (KMA, JAH, MSH, unpublished data). Sequences were checked for contamination by comparing to GenBank blast libraries using the sequence search tool in Geneious, and by translating sequences into amino acids where appropriate and searching for internal stop codons. All contaminated sequences were discarded. Sequence editing and alignment also occurred in Geneious, using the MAAFT plugin (Katoh et al. 2002) for all genes, with default settings. Due to the phylogenetic breadth of the dataset, the 12S gene alignment had many regions that were difficult to align. Therefore, the program Gblocks (Castresana 2000; Talavera & Castresana 2007) was used to remove ambiguously aligned sites for the 12S alignment. The web tool was chosen for this task (online at http://molevol.cmima.csic.es/castresana/Gblocks_server.html), and the settings allowed for gaps and larger blocks. The alignment files for each gene region are included as Supplementary Files 1–4 (online at http://dx.doi.org/10.1636/JoA-S-17-101.S1 through dx.doi.org/10.1636/JoA-S-17-101.S4). Phylogenetic trees were built for each alignment separately, using the RAxML plug-in (version 7.2.8) (Stamatakis 2006) in Geneious using the default settings with 100 bootstrap replicates and the GTR+G nucleotide substitution model. A concatenated alignment was also built and analyzed using RAxML, with partitions relating to the different gene regions.

Molecular taxonomy: All bases that were fixed within a species, and unique to that species (single pure characters, following Jörger & Schrödl 2013), were identified for each gene using the R package 'Spider' (Brown et al. 2012). Species were compared to other described species in the genus *Paradraculoides*, including existing sequences on GenBank, and newly produced sequences in this study. To align these datasets, the MAAFT plugin in Geneious was used, with the default settings. The 12S alignment was not treated for non-informative sites for this analysis.

To report the molecular diagnoses, we detail the single pure characters derived from each gene using 'Spider' (Table 2). The characters are reported as the base pair number, and the unique base for the species at that site. The base pair number is relative to the start of sequences derived from one specimen, WAMT98698 (GenBank accession numbers: *COI*, MG913085; ITS2, MG913105; 28S, MG913033; 12S, MG913012), and for the rRNA genes, also include indels. Therefore, interpretation of these diagnostic bases should ensure the recreation of the original alignments (Supplementary Files 1–4, online at http://dx.doi.org/10.1636/JoA-S-17-101.S1 through dx.doi.org/10.1636/JoA-S-17-101.S4).

RESULTS

Molecular phylogenetic analysis.—Individual gene trees and the concatenated gene tree (Figs. 2A–2D) supported the existence of seven new lineages, each of which is distinct from the described *Paradraculoides* species. However, the topologies of these different trees were incongruent and had low bootstrap support for deeper nodes. In each gene tree, the Robe River Valley species found to the north of the study area (i.e., *P. anachoretus*, *P. bythius*, *P. gnophicola* and *P. kryptus*) all formed clades with high support (Figs. 2A–2D). The new species formed three groups in the phylogenies. The most southern group included *P. confusus*, *P. trinity* and *P. catho*, which was recovered in all trees (Figs. 2A–B, 2D). The most northern group comprised *P. affinis*, *P. obrutus* and *P. celatus*, which was recovered in the concatenated, 12S and ITS2 trees (Figs. 2C–D). The final group contained a single species, *P. cochranus*, the farthest north of the study species, and was most closely related to those species in the adjacent Robe River Valley (*P. anachoretus*, *P. bythius*, *P. gnophicola* and *P. kryptus*) in the concatenated, 12S and ITS2 trees (Figs. 2A, 2C–D). *Paradraculoides eremius*, which is found farthest east in the study area, had an uncertain relationship to these main clades.

The seven molecular groups are distinct from each other, as well as from the previously described species of *Paradraculoides*, which are located north of the present study area (Harvey et al. 2008). Four of these clades are represented by adult male specimens, and the morphological details of the flagellum (shape and setal positions) were sufficiently distinct from each other to warrant their recognition as different species. Two of the other three clades were represented only by adult females and juveniles, and the seventh clade was represented by a single juvenile, and none were morphologically distinct from females or juveniles of the other three clades. As advocated by Cook et al. (2010), we believe that sufficient evidence is available to justify treating each clade as a distinct species, and here formally name them and diagnose them (Table 2). Our hypothesis can be tested by continued sampling at these sites to attempt to recover an adult male.

Despite producing molecular data for all proposed species, we have not applied species delimitation methods to these data. With only two independent markers with sufficient genetic variation to resolve species and populations (the linked mtDNA loci, and ITS2), the more rigorous delimitation

Table 2.—Unique ("pure") nucleotide substitutions for each of the seven new species of *Paradraculoides*. Details for notation are described in Methods.

Taxon	Gene region	*n*	Unique substitutions
Paradraculoides affinis sp. nov.	*COI*	39	403 (C), 557 (T)
	ITS2	24	199 (A)
	28S	9	Nil
	12S	7	4 (G), 36 (A), 58 (T), 72 (G), 95 (A), 135 (G), 136 (T), 184 (A), 185 (A), 199 (G), 239, T), 298 (G), 358 (C)
Paradraculoides catho sp. nov.	*COI*	2	184 (G), 583 (C), 735 (T), 754 (T)
	ITS2	2	159 (T), 166 (A)
	28S	2	636 (T), 937 (T)
	12S	1	14 (A), 40 (C), 51 (G), 52 (G), 109 (G), 131 (T), 132 (T), 188 (A), 189 (A), 190 (T), 201 (A), 250 (A), 251 (A), 254 (G), 259 (A), 262 (G), 281 (G)
Paradraculoides celatus sp. nov.	*COI*	1	86 (G), 205 (T), 241 (C), 301 (T), 370 (T), 388 (C), 625 (C), 799 (C)
	ITS2	1	Nil
	28S	1	Nil
	12S	1	4 (T), 22 (A), 197 (A), 202 (G), 239 (G), 326 (A), 333 (A)
Paradraculoides cochranus sp. nov.	*COI*	7	Nil
	ITS2	6	133 (A), 235 (T), 263 (G), 279 (G)
	28S	0	
	12S	0	
Paradraculoides confusus sp. nov.	*COI*	1	325 (C), 518 (G), 535 (C), 565 (C), 574 (C)
	ITS2	1	251 (T), 271 (T)
	28S	1	940 (A)
	12S	0	
Paradraculoides obrutus sp. nov.	*COI*	2	85 (T), 154 (T), 187 (C), 274 (C), 310 (T), 485 (T), 562 (C), 631 (T), 722 (G), 724 (A), 764 (T), 853 (C)
	ITS2	2	199 (G), 281 (C)
	28S	2	755 (A)
	12S	0	
Paradraculoides trinity sp. nov.	*COI*	4	292 (T), 389 (A), 474 (T), 785 (T)
	ITS2	2	192 (A), 277 (G)
	28S	1	469 (T), 647 (T), 661 (G)
	12S	0	

methods that require accurate guide-trees are not appropriate (Carstens et al. 2013). In addition, significant evidence has accumulated to suggest that single locus methods are inadequate (Dupuis et al. 2012). The proposed species may be further split by more molecular or morphological data, especially within species where we have detected genetic divergence (e.g., as in *P. cochranus*).

Problematic specimens.—Most specimens showed high location fidelity, such that those collected from each landform exhibited a distinct genetic signature. Three specimens, however, confounded this pattern with the molecular analysis placing them in different clades than would be expected on the basis of the locality data provided with the specimen:

T93208, juvenile from Trinity Bore, bore TBRC008; sequenced as *P. cochranus*.
T93217, female from Cane and Upper Cane, bore UCRC072; sequenced as *P. cochranus*.
T54123, female from Jewel and Cochrane Bore RNRC189; sequenced as *P. affinis*.

This pattern was detected using *COI* data for all three specimens, and for T93217 also with ITS2. We can only offer four explanations for this result. The first is that the specimens are indeed isolated genetic relicts found in different geographically disjunct subterranean environments to all other specimens of the same genetic clades. The second is that the samples were inadvertently mislabeled in the field or the laboratory. The third is that field samples were not completely sealed during transport from the study area to the laboratory, and the live specimens moved from one Tullgren funnel trap to another during the extraction process. The fourth is that contamination occurred in the molecular laboratory. Scenarios two, three and four seem considerably more likely than the first and we therefore exclude these three specimens from the maps and the list of material examined.

SYSTEMATICS

Family Hubbardiidae Cook, 1899
Subfamily Hubbardiinae Cook, 1899
Paradraculoides Harvey, Berry, Edward & Humphreys, 2008

Paradraculoides Harvey, Berry, Edward & Humphreys 2008: 185.

Type species.—*Paradraculoides kryptus* Harvey, Berry, Edward & Humphreys, 2008, by original designation.

Remarks.—The genus *Paradraculoides* was hypothesized as the sister-group to *Draculoides* by Harvey et al. (2008), a result confirmed by Clouse et al. (2017) in a multi-gene analysis of schizomids. Harvey et al. (2008) described four species of

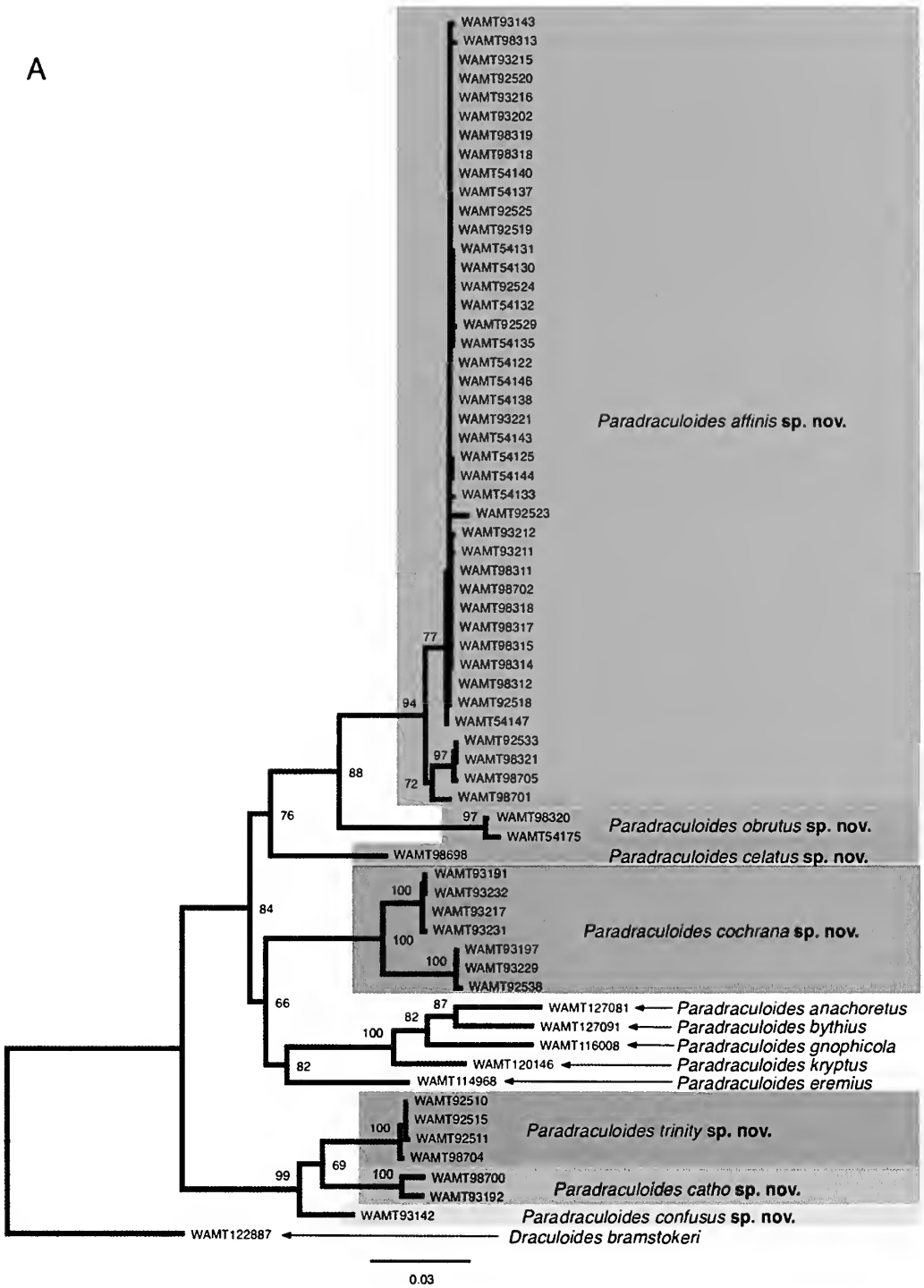

Figure 2A.—Maximum likelihood phylogeny of *Paradraculoides*, using the concatenated dataset (12S, *COI*, ITS2 and 28S). Bootstrap values are presented for all nodes. See 'Methods' for more details.

Paradraculoides, and recognized a fifth unnamed species which was not named due to the lack of adult specimens. These species were located along the Robe River valley, an ancient paleo-drainage system containing a number of Channel Iron Deposit mesas (Ramanaidou et al. 2003), each with an endemic suite of troglobites. *Paradraculoides* was defined by the presence of three macrosetae on tergite II and the lack of a laterally compressed male flagellum (Harvey et al. 2008). The geographically farthest species, *P. eremius* Abrams & Harvey, 2015, was recently described from Bungaroo, located 30 km south-east of the Robe River valley (Abrams & Harvey 2015).

While the new species described below generally fit the generic diagnosis, one species, *P. confusus*, has three pairs of macrosetae on tergite II like other species of *Paradraculoides*, but has a laterally compressed male flagellum, like species of *Draculoides* (Harvey 1992, 2001; Harvey et al. 2008). This

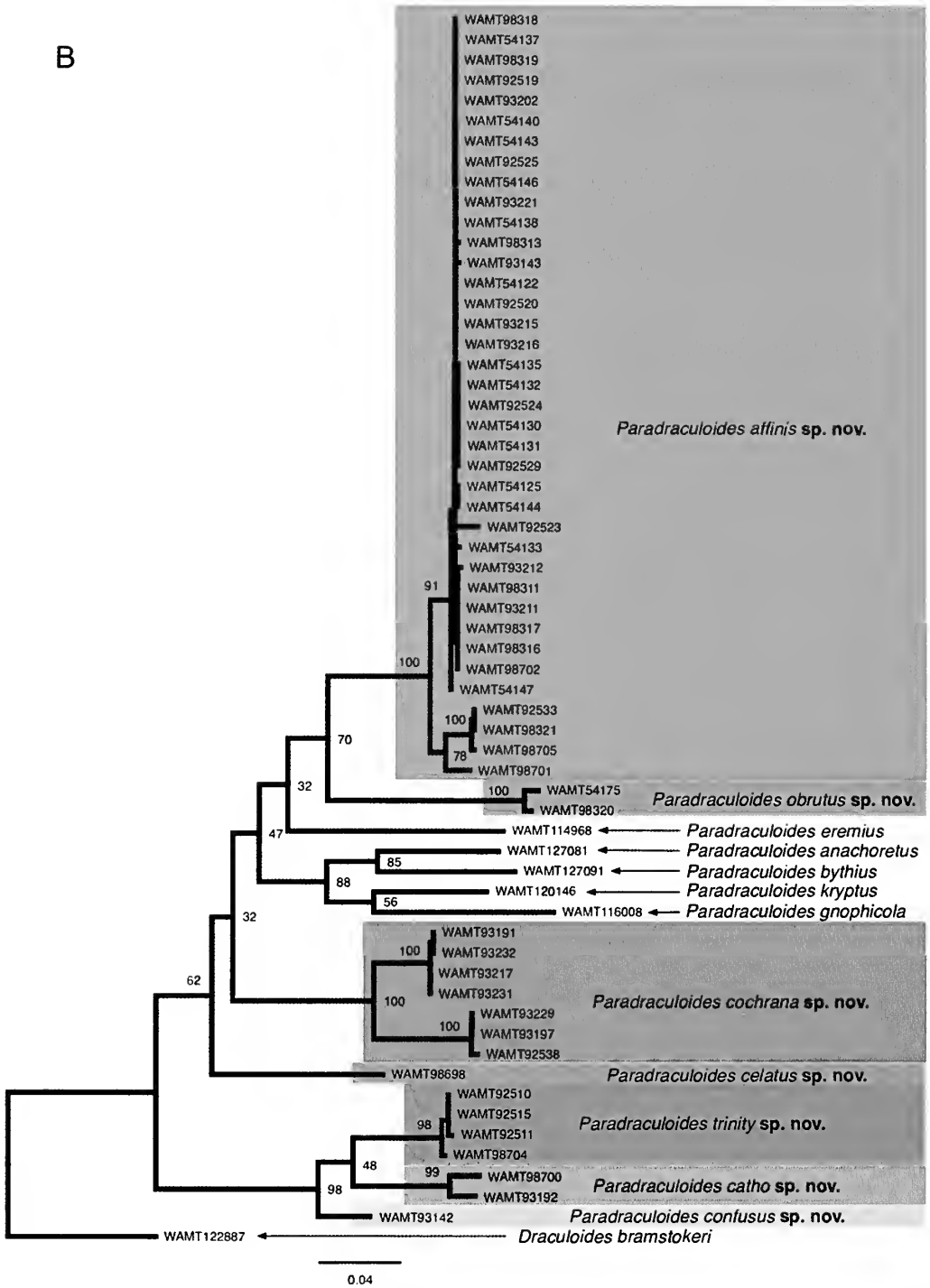

Figure 2B.—Maximum likelihood phylogeny of *Paradraculoides*, using the *CO1* data set. Bootstrap values are presented for all nodes. See 'Methods' for more details.

species casts considerable doubt on whether *Paradraculoides* should be retained as a distinct genus. In addition, Clouse et al. (2017) reported a similar result in a multi-gene analysis of schizomids, but we defer any formal synonymy as we are currently undertaking a thorough large-scale review of both genera, which are particularly diverse across the Pilbara bioregion of Western Australia.

Paradraculoides affinis sp. nov.
http://zoobank.org:8080/NomenclaturalActs/A9243824-BE1B-4906-88DB-DA80434FF7EF
(Figs. 3–5)

Type material.—*Holotype male*. AUSTRALIA: *Western Australia*: Cane and Upper Cane [River], 60.6 km S. of

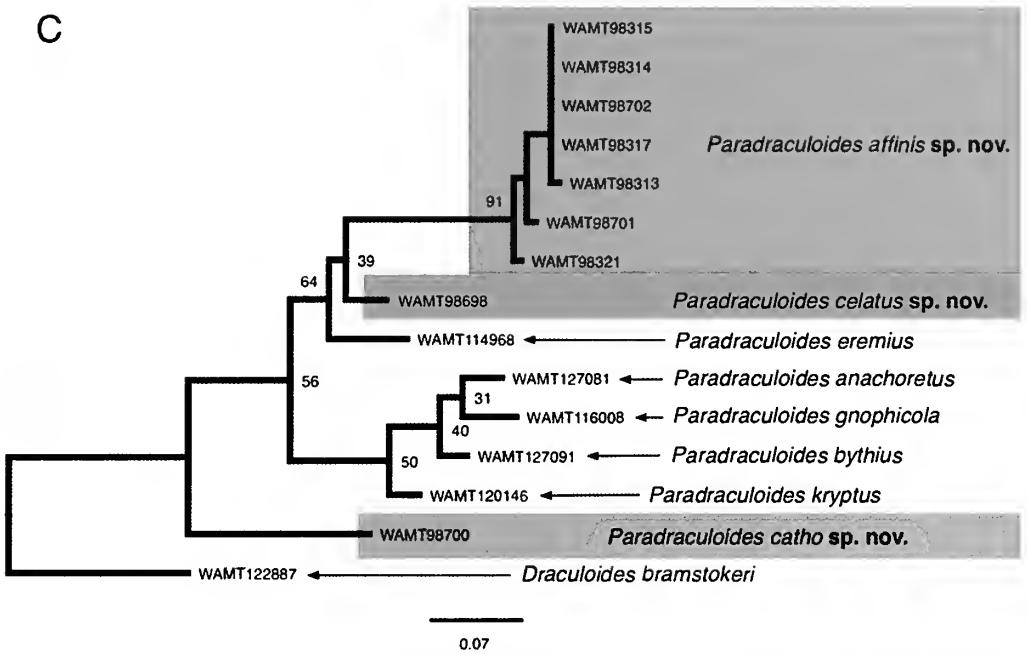

Figure 2C.—Maximum likelihood phylogeny of *Paradraculoides*, using the 12S data set. Bootstrap values are presented for all nodes. See 'Methods' for more details.

Pannawonica, 22°11′32″S, 116°16′31″E, 25–28 November 2008, troglofauna trap, 20 m, J. Cairnes, M. Menz (Biota Environmental Sciences, UCRC040P5T2–3) (WAM T93211) (DNA: *COI*, ITS2).

Paratypes. AUSTRALIA: *Western Australia*: 1 ♀, Cardo Bore North, 55.7 km S. of Pannawonica, 22°08′24″S, 116°14′06″E, 15–21 August 2008, troglofauna trap, 15 m, J. Alexander, T. Sachse (Biota Environmental Sciences, CBRC091P3T1–2) (WAM T93141); 1 ♀, Upper Cane, 60.6 km S. of Pannawonica, 22°11′32″S, 116°16′31″E, 7–10 July 2009, troglofauna trap, 30 m, J. Cairnes, D. Keirle (Biota Environmental Sciences, UCRC040P8T3–5) (WAM T98702) (DNA: *COI*).

Other material examined.—AUSTRALIA: *Western Australia*: 1 ♀, Cardo Bore North, 54.9 km S. of Pannawonica, 22°08′15″S, 116°13′59″E (WAM T92533) (DNA: *COI*); 1 ♀, Cardo Bore North, 61.2 km S. of Pannawonica, 22°11′29″S, 116°13′38″E (WAM T93224); 1 ♀, Cardo Bore North, 62.3 km S. of Pannawonica, 22°11′53″S, 116°12′18″E (WAM T93225); 1 ♀, Cardo Bore East, 61 km S. of Pannawonica, 22°11′28″S, 116°14′38″E (WAM T93199); 1 ♂, Cane and Upper Cane [River], 60.3 km S. of Pannawonica, 22°11′25″S, 116°16′07″E, 15–21 August 2008, troglofauna trap, 10 m, J. Alexander, T. Sachse (Biota Environmental Sciences, UCRC051P3T1–3) (WAM T92523) (DNA: *COI*); 1 ♂, Cane and Upper Cane Bores, site UCRC003, 22°11′24.81″S, 116°16′38.78″E, 17 January 2008, troglofauna trap, J. Alexander, T. Sasche (Biota Environmental Sciences, UCRC003P2T1–1) (WAM T54142); 1 ♂ (body lost), Cardo Bore North, 55.7 km S. of Pannawonica, 22°08′26″S, 116°13′48″E, 25–28 November 2008, troglofauna trap, 15 m, J. Cairnes, M. Menz (Biota Environmental Sciences, CBRC099P5T1–3) (WAM T93223); 1 juvenile, Cane and Upper Cane Bores, site UCRC069, 22°11′44.04″S, 116°15′35.26″E, 17 January 2008, troglofauna trap, J. Alexander, T. Sachse (Biota Environmental Sciences, UCRC069P2T3–1) (WAM T54132) (DNA: *COI*, ITS2); 1 juvenile, Cane and Upper Cane Bores, site UCRC062, 22°11′42.91″S, 116°15′16.30″E, 17 January 2008, troglofauna trap, J. Alexander, T. Sachse (Biota Environmental Sciences, UCRC062P2T3–1) (WAM T54147) (DNA: *COI*); 1 juvenile, Cane and Upper Cane Bores, site UCRC040, 22°11′32.16″S, 116°16′31.27″E, 17 January 2008, troglofauna trap, J. Alexander, T. Sachse (Biota Environmental Sciences, UCRC040P2T1–1) (WAM T54135) (DNA: *COI*); 1 ♂, Cane and Upper Cane Bores, site UCRC003, 22°11′24.81″S, 116°16′38.78″E, 17 January 2008, troglofauna trap, J. Alexander, T. Sachse (Biota Environmental Sciences, UCRC003P2T2–1) (WAM T54141); 1 juvenile, Upper Cane, 60.6 km S. of Pannawonica, 22°11′32″S, 116°16′31″E, 7–10 July 2009, troglofauna trap, 20 m, J. Cairnes, D. Keirle (Biota Environmental Sciences, UCRC040P8T2–4) (WAM T98703); 1 ♀, Cane and Upper Cane [River], 60.9 km S. of Pannawonica, 22°11′32″S, 116°16′07″E, 25–28 November 2008, troglofauna trap, 45 m, J. Cairnes, M. Menz (Biota Environmental Sciences, UCRC053P5T3–2) (WAM T93221) (DNA: *COI*, ITS2); 1 ♀, Cane and Upper Cane [River], 60.6 km S. of Pannawonica, 22°11′36″S, 116°15′55″E, 25–28 November 2008, troglofauna trap, 40 m, J. Cairnes, M. Menz (Biota Environmental Sciences, UCRC076P5T3–3) (WAM T93215) (DNA: *COI*); 1 ♂, 1 ♀, 1 juvenile, Cane and Upper Cane [River], 60.6 km S. of Pannawonica, 22°11′32″S, 116°16′31″E, 25–28 November 2008, troglofauna trap, 30 m, J. Cairnes, M. Menz (Biota Environmental Sciences, UCRC040P5T3–3) (WAM T93212) (DNA: *COI*, ITS2); 1 ♂, 1 juvenile, Cane and Upper Cane [River], 60.6 km S. of Pannawonica, 22°11′32″S, 116°16′31″E, 25–28 November 2008, troglofauna trap, 10 m, J. Cairnes, M. Menz (Biota

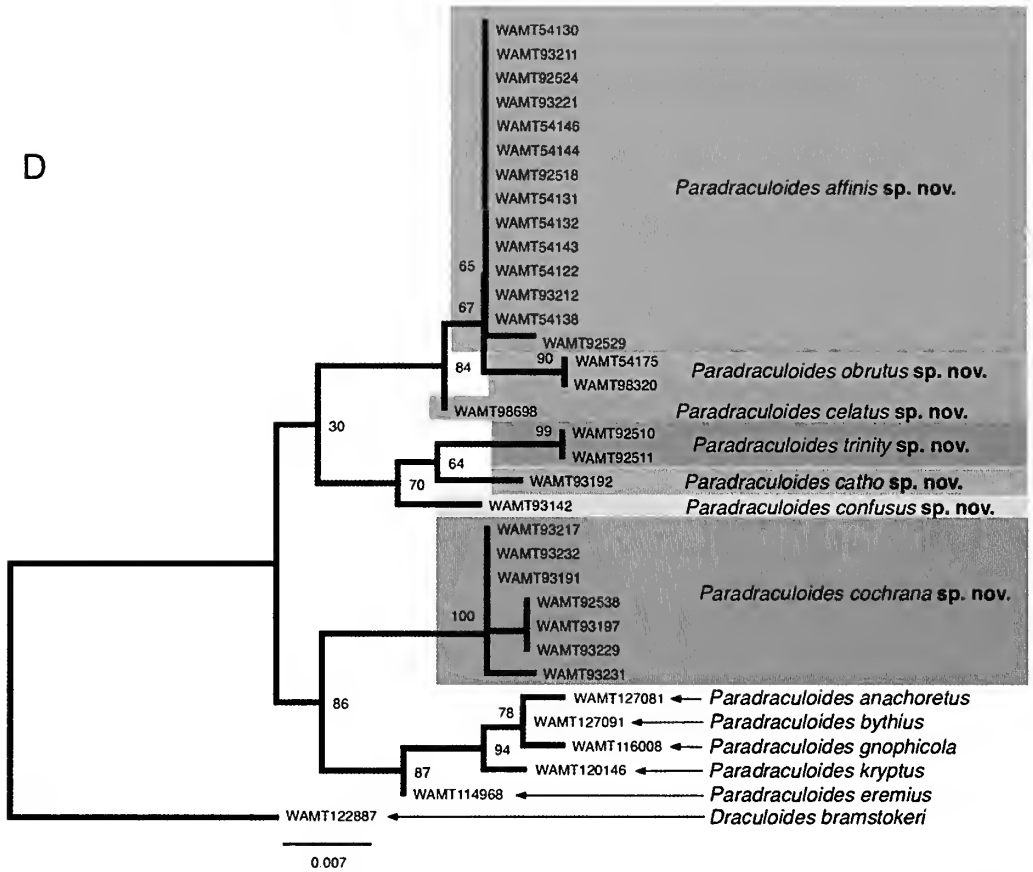

Figure 2D.—Maximum likelihood phylogeny of *Paradraculoides*, using the ITS2 data set. Bootstrap values are presented for all nodes. See 'Methods' for more details.

Environmental Sciences, UCRC040P5T1–4) (WAM T93214); 1 ♀, 1 juvenile, Cane and Upper Cane [River], 60.9 km S. of Pannawonica, 22°11′39″S, 116°15′33″E, 25–28 November 2008, troglofauna trap, 45 m, J. Cairnes, M. Menz (Biota Environmental Sciences, UCRC029P5T3–2) (WAM T93216) (DNA: *COI*); 2 ♀, Cane and Upper Cane, 61 km S. of Pannawonica, 22°11′35″S, 116°15′11″E, 13–16 October 2008, troglofauna trap, 25 m, G. Humphreys, M. Menz (Biota Environmental Sciences, UCRC021P4T2–1) (WAM T93202) (DNA: *COI*); 1 ♀, Upper Cane, 60.6 km S. of Pannawonica, 22°11′32″S, 116°16′31″E, 12–15 September 2009, troglofauna trap, 10 m, D. Kamien (Biota Environmental Sciences, UCRC040P7T1–1) (WAM T98315); 1 ♀, Upper Cane, 60.6 km S. of Pannawonica, 22°11′36″S, 116°15′55″E, 12–15 September 2009, troglofauna trap, 10 m, D. Kamien (Biota Environmental Sciences, UCRC076P7T1–1) (WAM T98318) (DNA: *COI*); 1 ♀, Upper Cane, 60.6 km S. of Pannawonica, 22°11′32″S, 116°16′31″E, 12–15 September 2009, troglofauna trap, 30 m, D. Kamien (Biota Environmental Sciences, UCRC040P7T3–1) (WAM T98316) (DNA: *COI*); 1 ♀, Upper Cane, 60.6 km S. of Pannawonica, 22°11′32″S, 116°16′31″E, 12–15 September 2009, troglofauna trap, 10 m, D. Kamien (Biota Environmental Sciences, UCRC040P7T1–3) (WAM T98317) (DNA: *COI*); 1 juvenile, Upper Cane, 60 km S. of Pannawonica, 22°11′19″S, 116°16′03″E, 12–15 September 2009, troglofauna trap, 10 m, D. Kamien (Biota Environmental Sciences, UCRC093P7T2–1) (WAM T98319) (DNA: *COI*); 1 ♀, Upper Cane, 61.6 km S. of Pannawonica, 22°11′30″S, 116°15′04″E, 12–15 September 2009, troglofauna trap, 35 m, D. Kamien (Biota Environmental Sciences, UCRC055P7T3–1) (WAM T98314); 1 ♀, Upper Cane, 60.6 km S. of Pannawonica, 22°11′36″S, 116°15′55″E, 12–15 September 2009, troglofauna trap, 40 m, D. Kamien (Biota Environmental Sciences, UCRC076P7T3–5) (WAM T98311); 1 ♂, Upper Cane, 60.6 km S. of Pannawonica, 22°11′32″S, 116°16′31″E, 12–15 September 2009, troglofauna trap, 20 m, D. Kamien (Biota Environmental Sciences, UCRC040P7T2–1) (WAM T98313) (DNA: *COI*); 2 ♀, Cardo Bore, 56.2 km S. of Pannawonica, 22°08′31″S, 116°13′15″E, 12–15 September 2009, troglofauna trap, 15 m, D. Kamien (Biota Environmental Sciences, CBRC192P7T1–1) (WAM T98321) (DNA: *COI*); 1 ♀, Upper Cane, 60.9 km S. of Pannawonica, 22°11′39″S, 116°15′53″E, 12–15 September 2009, troglofauna trap, 10 m, D. Kamien (Biota Environmental Sciences, UCRC029P7T1–2) (WAM T98312); 1 juvenile, Cane and Upper Cane Bores, site UCRC064, 22°11′46.38″S, 116°15′21.23″E, 24 October 2007, troglofauna trap, D. Kamien, J. Alexander (Biota Environmental Sciences, UCRC064T2–2) (WAM T54125) (DNA: *COI*); 1 ♂, Cane and Upper Cane Bores, site UCRC042, 22°11′27.46″S, 116°16′27.76″E, 17 January 2008, troglofauna trap, J. Alexander, T. Sachse (Biota Environmental Sciences, UCRC042P2T3–1) (WAM T54145); 1 ♀, Cane and Upper Cane Bores, site UCRC076, 22°11′35.58″S, 116°15′54.89″E, 24 October 2007, troglofauna trap, D.

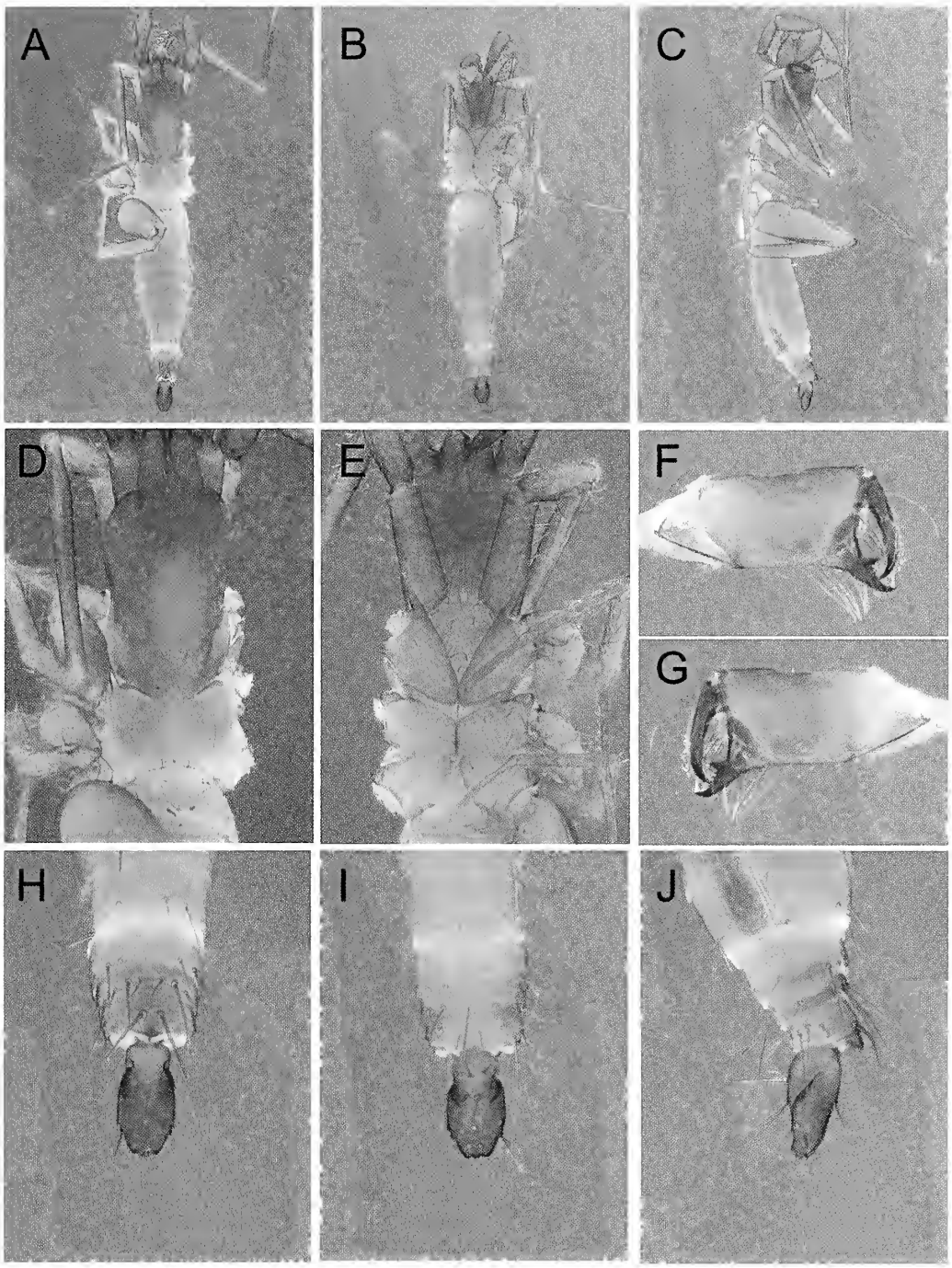

Figure 3.—*Paradraculoides affinis* sp. nov., holotype male (WAM T93211): A. Body, dorsal; B. Body, ventral; C. Body, lateral; D. Cephalothorax, dorsal; E. Cephalothorax, ventral; F. Left chelicera, prolateral; G. Left chelicera, retrolateral; H. Flagellum, dorsal; I. Flagellum, ventral; J. Flagellum, lateral. Abbreviations: dm1, 4 (dorso-median 1, 4), dl1, 3 (dorso-lateral 1, 3), vm1, 2, 3, 5 (ventro-median 1, 2, 3, 5), vl1, 2 (ventro-lateral 1, 2).

Kamien, J. Alexander (Biota Environmental Sciences, UCRC076T1–1) (WAM T54122) (DNA: *COI*, ITS2); 2 ♀, Cane and Upper Cane Bores, site UCRC029, 22°11′38.66″S, 116°15′52.71″E, 24 October 2007, troglofauna trap, D. Kamien, J. Alexander (Biota Environmental Sciences, UCRC029T3–1) (WAM T54130) (DNA: *COI*, ITS2); 1 juvenile, Cane and Upper Cane Bores, site UCRC021, 22°11′35.31″S, 116°15′10.72″E, 24 October 2007, troglofauna trap, D. Kamien, J. Alexander (Biota Environmental Sciences, UCRC021T3–1) (WAM T54131) (DNA: *COI*, ITS2); 1 ♀, 3 juveniles, Cane and Upper Cane Bores, site UCRC072, 22°11′38.06″S, 116°15′41.99″E, 24 October 2007, troglofauna trap, D. Kamien, J. Alexander (Biota Environmental Sciences, UCRC072T3–1) (WAM T54124); 1 juvenile, Cane and Upper Cane Bores, site UCRC069, 22°11′44.04″S, 116°15′35.26″E, 24 October 2007, troglofauna trap, D. Kamien, J. Alexander (Biota Environmental Sciences, UCRC069T2–1) (WAM T54127); 1 ♂, 2 juveniles, Cane and Upper Cane Bores, site

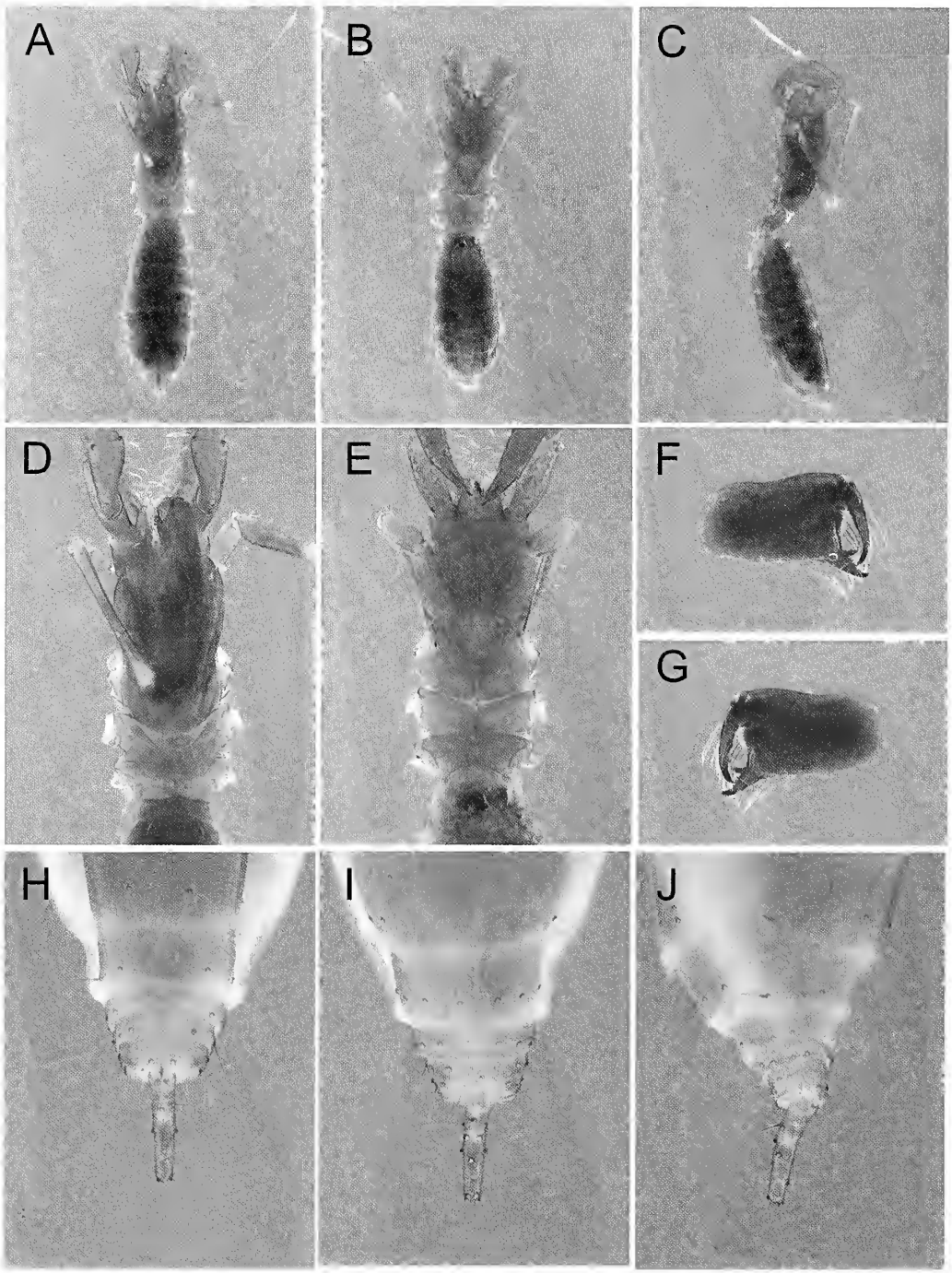

Figure 4.—*Paradraculoides affuis* sp. nov.: A–G, paratype female (WAM T93141): A. Body, dorsal; B. Body, ventral; C. Body, lateral; D. Cephalothorax, dorsal; E. Cephalothorax, ventral; F. Left chelicera, prolateral; G. Left chelicera, retrolateral. H–J, paratype female (WAM T98702): H. Flagellum, dorsal; I. Flagellum, ventral; J. Flagellum, lateral. Abbreviations: dm1, 4 (dorso-median 1, 4), dl1, 3 (dorso-lateral 1, 3), vm1, 2, 3, 5 (ventro-median 1, 2, 3, 5), vl1, 2 (ventro-lateral 1, 2).

UCRC069, 22°11′44.04″S, 116°15′35.26″E, 24 October 2007, troglofauna trap, D. Kamien, J. Alexander (Biota Environmental Seienees, UCRC069T3–1) (WAM T54139); 1 ♀, Cane and Upper Cane Bores, site UCRC076, 22°11′35.58″S, 116°15′54.89″E, 24 October 2007, troglofauna trap, D. Kamien, J. Alexander (Biota Environmental Seienees, UCRC076T2–1) (WAM T54138) (DNA: *COI*, ITS2); 1 juvenile, Upper Cane, 60.1 km S. of Pannawonica, 22°11′09″S, 116°16′03″E, 8 September 2009, troglofauna trap, 30 m, P. Runham, J. Alexander (Biota Environmental Sciences, PH16P8T3–3) (WAM T98699); 1 juvenile, Upper Cane, 60.1 km S. of Pannawoniea, 22°11′09″S, 116°16′03″E, 8 September 2009, troglofauna trap, 10 m, P. Runham, J. Alexander (Biota Environmental Seienees, PH16P8T1–1) (WAM T98701) (DNA: *COI*); 1 juvenile, Upper Cane, 60.1 km S. of Pannawoniea, 22°11′09″S, 116°16′03″E, 8 September

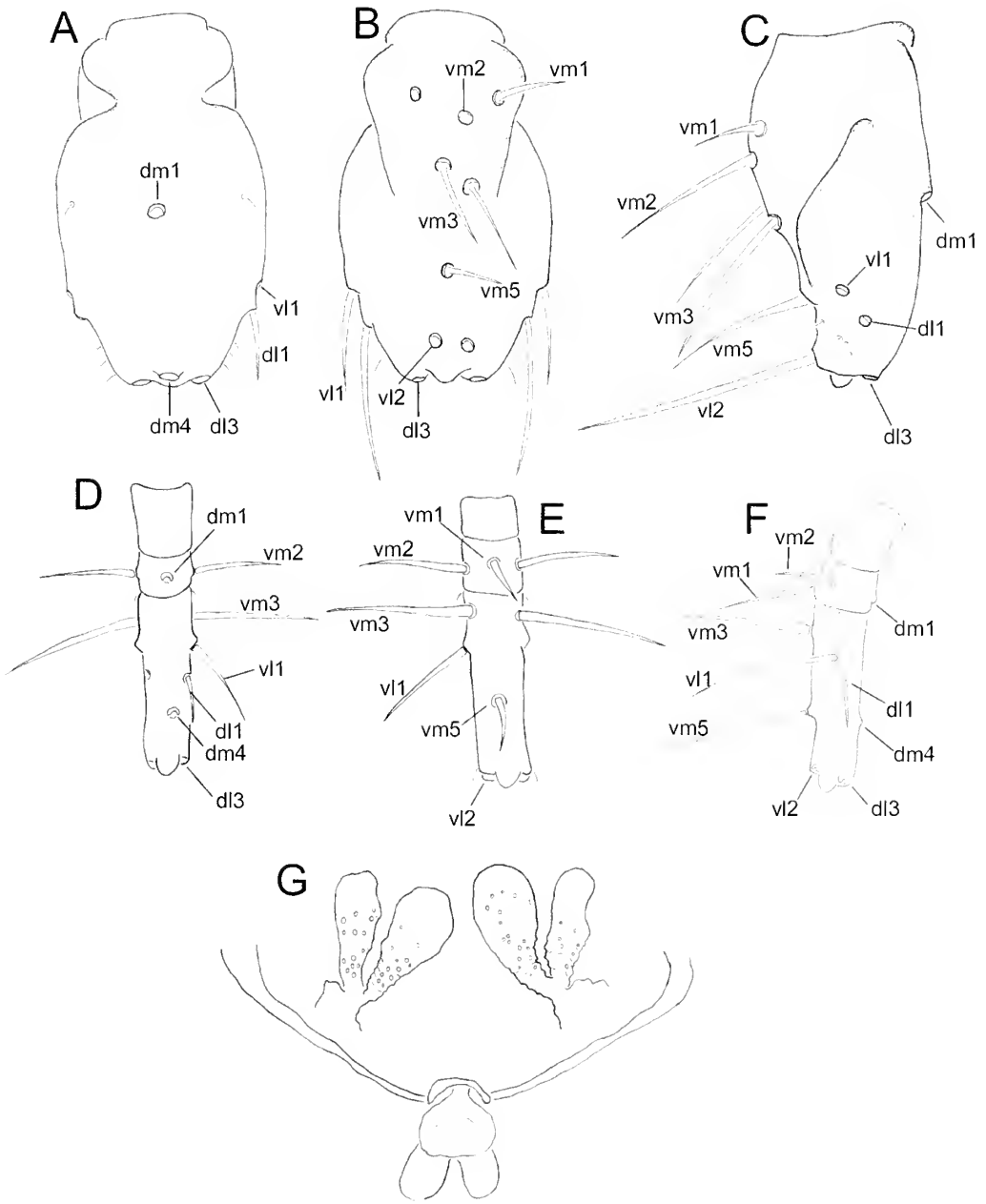

Figure 5.—*Paradraculoides affinis* sp. nov.: A–C, holotype male (WAM T93211): A. Flagellum, dorsal; B. Flagellum, ventral; C. Flagellum, lateral. D–G, paratype female (WAM T93141): D. Flagellum, dorsal; E. Flagellum, ventral; F. Flagellum, lateral; G. Spermathecae, ventral. Abbreviations: dm1, 4 (dorso-median 1, 4), dl1, 3 (dorso-lateral 1, 3), vm1, 2, 3, 5 (ventro-median 1, 2, 3, 5), vl1, 2 (ventro-lateral 1, 2).

2009, troglofauna trap, 20 m, P. Runham, J. Alexander (Biota Environmental Sciences, PH16P8T2–1) (WAM T98705) (DNA: *COI*); 1 ♀, Cane and Upper Cane [River], 60.2 km S. of Pannawonica, 22°11′27″S, 116°16′28″E, 15–21 August 2008, troglofauna trap, 20 m, J. Alexander, T. Sachse (Biota Environmental Sciences, UCRC042P3T2–1) (WAM T92525) (DNA: *COI*); 1 ♀, Cane and Upper Cane [River], 60.5 km S. of Pannawonica, 22°11′43″S, 116°15′16″E, 15–21 August 2008, troglofauna trap, 35 m, J. Alexander, T. Sachse (Biota Environmental Sciences, UCRC062P3T3–1) (WAM T92529) (DNA: *COI*, ITS2); 1 ♀, 2 juveniles, Cane and Upper Cane [River], 60.2 km S. of Pannawonica, 22°11′27″S, 116°16′28″E, 15–21 August 2008, troglofauna trap, 10 m, J. Alexander, T. Sachse (Biota Environmental Sciences, UCRC042P3T1–1) (WAM T92528); 1 ♀, Cane and Upper Cane [River], 61.0 km S. of Pannawonica, 22°11′35″S, 116°15′11″E, 15–21 August 2008, troglofauna trap, 10 m, J. Alexander, T. Sachse (Biota Environmental Sciences, UCRC021P3T1–3) (WAM T92521); 2 ♂, 2 ♀, 1 juvenile, Cane and Upper Cane [River], 60.6 km S. of Pannawonica, 22°11′32″S, 116°16′31″E, 15–21 August 2008, troglofauna trap, 30 m, J. Alexander, T. Sachse (Biota Environmental Sciences, UCRC040P3T3–1) (WAM T92522); 1 ♀, Cane and Upper Cane [River], 60.8 km S. of Pannawonica, 22°11′33″S, 116°15′49″E, 15–21 August 2008, troglofauna trap, 45 m, J. Alexander, T. Sachse (Biota Environmental Sciences, UCRC078P3T3–1) (WAM T92519)

(DNA: *COI*); 1 ♀, Cane and Upper Cane [River], 60.6 km S. of Pannawonica, 22°11′33″S, 116°16′31″E, 15–21 August 2008, troglofauna trap, 10 m, J. Alexander, T. Sachse (Biota Environmental Sciences, UCRC040P3T1–1) (WAM T92518) (DNA: ITS2); 1 ♀, Cane and Upper Cane [River], 61.5 km S. of Pannawonica, 22°11′44″S, 116°15′35″E, 15–21 August 2008, troglofauna trap, 15 m, J. Alexander, T. Sachse (Biota Environmental Sciences, UCRC069P3T1–1) (WAM T92524) (DNA: *COI*, ITS2); 1 ♀, Cane and Upper Cane [River], 60.3 km S. of Pannawonica, 22°11′25″S, 116°16′07″E, 15–21 August 2008, troglofauna trap, 20 m, J. Alexander, T. Sachse (Biota Environmental Sciences, UCRC051P3T2–2) (WAM T92520) (DNA: *COI*); 1 ♀, Cane and Upper Cane [River], 61.5 km S. of Pannawonica, 22°11′44″S, 116°15′35″E, 15–21 August 2008, troglofauna trap, 30 m, J. Alexander, T. Sachse (Biota Environmental Sciences, UCRC069P3T2–1) (WAM T93143) (DNA: *COI*); 1 juvenile, Cane and Upper Cane Bores, site UCRC029, 22°11′38.66″S, 116°15′52.71″E, 17 January 2008, troglofauna trap, J. Alexander, T. Sachse (Biota Environmental Sciences, UCRC029P2T1–1) (WAM T54133) (DNA: *COI*); 1 juvenile, Cane and Upper Cane Bores, site UCRC051, 22°11′24.48″S, 116°16′06.75″E, 17 January 2008, troglofauna trap, J. Alexander, T. Sachse (Biota Environmental Sciences, UCRC051P2T3–1) (WAM T54143) (DNA: *COI*, ITS2); 1 juvenile, Cane and Upper Cane Bores, site UCRC076, 22°11′35.58″S, 116°15′54.89″E, 17 January 2008, troglofauna trap, J. Alexander, T. Sachse (Biota Environmental Sciences, UCRC076P2T2–2) (WAM T54140) (DNA: *COI*); 1 juvenile, Cane and Upper Cane Bores, site UCRC053, 22°11′31.99″S, 116°16′06.82″E, 17 January 2008, troglofauna trap, J. Alexander, T. Sasche (Biota Environmental Sciences, UCRC053P2T2–1) (WAM T54137) (DNA: *COI*); 1 juvenile, Cane and Upper Cane Bores, site UCRC051, 22°11′24.48″S, 116°16′06.75″E, 17 January 2008, troglofauna trap, J. Alexander, T. Sachse (Biota Environmental Sciences, UCRC051P2T2–1) (WAM T54146) (DNA: *COI*, ITS2); 1 juvenile, NE. of Red Hill, Cardo Bore, 22°11′48.36″S, 116°13′28.01″E, 17 January 2008, troglofauna trap, J. Alexander, T. Sachse (Biota Environmental Sciences, CBRC208P2T1–1) (WAM T54144) (DNA: *COI*, ITS2).

Etymology.—The specific name is an adjective referring to the similarity between this species and other species of the genus (*affinis*, Latin, related to, neighboring).

Diagnosis.—The shape of the male flagellum of *P. affinis* most closely resembles *P. bythius*, *P. cochranus*, *P. eremius* and *P. kryptus*, especially in the presence of a broad base. It differs from *P. cochranus* by the posterior position of dm4 (sub-distal in *P. cochranus*) and the close proximity of vl1 and dl1 such that vl1 is on the same level as vm5 (vl1 is anterior to vm5 in *P. bythius* and *P. kryptus*), from *P. bythius* and *P. kryptus* in the slightly broader flagellum, and from *P. eremius* by the raised antero-dorsal region of the flagellum, which is flat in *P. eremius*. Females differ from all other species of *Paradraculoides* by the comparatively wide separation of both setae vm3. Specimens of this species can be distinguished from all other species of *Paradraculoides* by the following combination of nucleotide substitutions: *COI* ($n = 39$): 403 (C), 557 (T); ITS2 ($n = 24$): 199 (A); 12S ($n = 7$): 4 (G), 36 (A), 58 (T), 72 (G), 95 (A), 135 (G), 136 (T), 184 (A), 185 (A), 199 (G), 239, T), 298 (G), 358 (C) (Table 2).

Description (adults).—*Color:* Yellow-brown; propeltidium somewhat darker; female darker with olive-grey tinge (Figs. 3A–C, 4A–C).

Cephalothorax: Propeltidium with 2 apical setae on anterior process and 2 + 2 + 2 setae; eye spots absent. Mesopeltidia separated. Anterior sternum with 14 (♂), 12 (♀) setae (including 2 sternapophysial setae); posterior sternum triangular with 6 setae.

Chelicera: Fixed finger with 2 large teeth plus 6 (♂), 5 (♀) smaller teeth between these; brush at base of fixed finger composed of 7 setae (G5A), each densely pilose in distal half; lateral surface with 3 large, lanceolate, terminally pilose setae (G1); internal face of chelicera with 4 (♂), 3 (♀) short whip-like setae (G4); movable finger serrula composed of 17 (♂), 18 (♀) long lamellae, blunt guard tooth present subdistally; 1 accessory tooth present at two-thirds from base of serrula.

Pedipalp: Without apophyses; trochanter with sharply produced ventro-distal extension, ventral margin with ca. 8 stout setae, without mesal spur; tarsus and tibia without spines; tarsal spur present; claw 0.52 (♂), 0.39 (♀) x length of tarsus.

Legs: Tarsus I with 6 tarsomeres; femur IV 3.18 (♂), 2.62 (♀) x longer than wide; baso-dorsal margin of femur IV produced at about a 90° angle.

Abdomen: Chaetotaxy of tergites I–IX: 2 macrosetae + 4 microsetae: 3 macrosetae + 6 microsetae (microsetae in column): 2: 2: 2: 2: 2: 2: 6.

Female genitalia: Two pairs of elongate spermathecae, each pair connected basally before connection with bursa (Fig. 5G), distally round and smooth; sparsely covered with small pores; gonopod short, distally bifurcate. *Flagellum.* Male: Dorsoventrally compressed (Figs. 3H–J, 4A–C); 2.06 x longer than broad; seta dm1 situated dorso-medially, slightly closer to anterior margin; seta dm4 situated at posterior margin; dl1 between dl3 and vl1; dl3 on posterior margin at similar level as dl1; vm2 situated on approximately same level as vm1; vm5 situated slightly closer to vl2 than to vm3; 1 pair of microsetae near anterior end, and three pairs between vl1 and dl3. Female: 5.50 x longer than broad; seta dm1 situated towards posterior end of second slightly more posterior than vm2; setae dl1 situated anterior to dm4, dm4 situated at four fifth length of flagellomere III; dl3 situated almost at posterior margin marginally more posterior than vl2, vm1 situated slightly more posterior than vm2, vm3 situated closer to vm1 than to vm5, vm5 halfway between vm3 and vl2, vl1 situated posterior to vm3 and anterior to dl1; 1 pair of microsetae baso-laterally on flagellomere III, 1 pair of microsetae laterally between dl3 and vl2.

Dimensions (mm): Holotype male (WAM T93211): Body length 3.15. Propeltidium 1.00/0.52. Chelicera 0.91. Flagellum 0.35/0.17. Pedipalp: trochanter 0.45, femur 0.47, patella 0.49, tibia 0.45, tarsus 0.25, claw 0.13, total excluding claw 2.11. Leg I: trochanter 0.37, femur 1.26, patella 1.59, tibia 1.26, metatarsus 0.38, tarsus 0.55, total 5.41. Leg IV: trochanter 0.32, femur 1.21/0.38, patella 0.42, tibia 0.88, metatarsus 0.79, tarsus 0.52, total 4.14.

Paratype female (WAM T93141): Body length 3.49. Propeltidium 1.15/0.61. Chelicera 0.72. Flagellum 0.33/0.06.

Pedipalp: trochanter 0.48, femur 0.54, patella 0.56, tibia 0.50, tarsus 0.35, claw 0.10, total excluding claw 2.43. Leg I: trochanter 0.35, femur 1.13, patella 1.36, tibia 1.08, metatarsus 0.33, tarsus 0.56, total 4.81. Leg IV: trochanter 0.31, femur 1.10/0.42, patella 0.44, tibia 0.79, metatarsus 0.71, tarsus 0.48, total 3.81.

Variation: Body length 2.31–3.64 (males, $n = 10$), 2.41–4.01 (females, $n = 10$); propeltidium 0.93–1.10/0.46-0.59 (males), 0.89–1.26/0.49–0.69 (females).

Remarks.—*Paradraculoides affinis* is known from several locations within two areas known as Cardo Bore and Cane and Upper Cane, situated at the western edge of the Hamersley Range, Western Australia (Fig. 1). The juvenile specimens listed above are associated with this species by locality and, in many cases, by sequence data (Figs. 2A–D).

Paradraculoides catho sp. nov.
http://zoobank.org:8080/NomenclaturalActs/04A7E113-EA1C-4BE3-B3CC-AF18FCA22571
(Figs. 6, 7)

Type material.—*Holotype female.* AUSTRALIA: *Western Australia*: Mt Stuart Station, Catho Well, 82.6 km S. of Pannawonica, 22°23′18″S, 116°15′11″E, 13–16 October 2008, troglofauna trap, 20 m, G. Humphreys, M. Menz (Biota Environmental Sciences, CWRC281P4T2–1) (WAM T93192) (DNA: *COI*, ITS2).

Other material examined.—AUSTRALIA: *Western Australia*: 1 juvenile, Mt Stuart Station, Catho Well, 22°24′36.35″S, 116°16′23.68″E (WAM T54126); 1 juvenile, collected with holotype (WAM T143826); 2 juveniles, Mt Stuart Station, Catho Well, 84.5 km S. of Pannawonica, 22°24′40″S, 116°16′10″E (WAM T92509); 1 juvenile, Catho Well, 84.7 km S. of Pannawonica, 22°24′28″S, 116°16′44″E (WAM T98700) (DNA: *COI*).

Etymology.—The specific epithet refers to the type locality, Catho Well, and is to be treated as a noun in apposition.

Diagnosis.—Males of *P. catho* are unknown, and although the setal arrangement on the female flagellum place this species closest to *P. trinity*, it is currently not possible to differentiate both species on morphology alone. Specimens of this species can be distinguished from all other species of *Paradraculoides* by the following combination of nucleotide substitutions: *COI* ($n = 2$): 184 (G), 583 (C), 735 (T), 754 (T); ITS2 ($n = 2$): 159 (T), 166 (A); 28S ($n = 2$): 636 (T), 937 (T); 12S ($n = 1$): 14 (A), 40 (C), 51 (G), 52 (G), 109 (G), 131 (T), 132 (T), 188 (A), 189 (A), 190 (T), 201 (A), 250 (A), 251 (A), 254 (G), 259 (A), 262 (G), 281 (G) (Table 2).

Description (adult female).—*Color:* Yellow-brown; propeltidium and pedipalps somewhat darker (Figs. 6A–C).

Cephalothorax: Propeltidium with 2 + 1 apical setae on anterior process and 2 + 1 (right) 2 + 2 setae; eye spots absent. Mesopeltidia separated. Anterior sternum with 11 setae (including 2 sternapophysial setae); posterior sternum triangular with 7 setae.

Chelicera: Fixed finger with two large teeth plus five smaller teeth between these; basal tooth with two short and very blunt teeth; brush at base of fixed finger composed of 9 setae (G5A), each densely pilose in distal half; lateral surface with 3 large, lanceolate, terminally pilose setae (G1); internal face of chelicera with 5 short whip-like setae (G4); movable finger serrula composed of 21 long lamellae, blunt guard tooth present subdistally; one large accessory tooth present at two-thirds from base of serrula accompanied by two smaller teeth, one basally one distally.

Pedipalp: Without apophyses; trochanter with sharply produced ventro-distal extension, ventral margin with ca. 10 stout setae, without mesal spur; tarsus and tibia without spines; tarsal spur present; claw 0.43 length of tarsus.

Legs: Tarsus I with 6 tarsomeres; femur IV 5.45 x longer than wide; baso-dorsal margin of femur IV produced at about a 90° angle.

Abdomen: Chaetotaxy of tergites I–IX: 2 macrosetae: 3 macrosetae (central somewhat smaller than laterals) + 6 microsetae (microsetae in column): 2: 2: 2: 2: 2: 2: 4.

Female genitalia: Two pairs of highly elongated spermathecae, sparsely covered with pores over their whole length; gonopod subrectangular with rounded corners (Fig. 7D).

Flagellum: 4.67 x longer than broad (Figs. 6H–J, 7A–C); seta dm1 situated towards posterior end of flagellomere II, at same level as vm2; setae dl1 situated anterior to dm4, dm4 situated at three quarters length of flagellomere III; dl3 situated at posterior margin more posterior than vl2, vm1 situated slightly more anterior than vm2, vm3 situated closer to vm1 than to vm5, vm5 slightly closer to vl2 than to vm3, vl1 situated posterior to vm3 and anterior to dl1; 1 pair of microsetae baso-laterally on flagellomere III, 1 pair of microsetae laterally between dl3 and vm5.

Dimensions (mm): Holotype female (WAM T93192): Body length 5.24. Propeltidium 1.52/0.83. Chelicera 1.14. Flagellum 0.42/0.09. Pedipalp: trochanter 0.71, femur 0.81, patella 0.88, tibia 0.90, tarsus 0.38, claw 0.16, total excluding claw 3.68. Leg I: trochanter 0.47, femur 1.81, patella 2.17, tibia 1.73 [metatarsus and tarsus both missing]. Leg IV: trochanter 0.50, femur 1.69/0.31, patella 0.77, tibia 1.29, metatarsus 1.13, tarsus 0.61, total 5.99.

Remarks.—*Paradraculoides catho* is known from three localities known as Catho Well, Western Australia (Fig. 1). All specimens were collected from bore holes using troglofauna traps, and were collected from sites less than 3.5 km apart.

Paradraculoides celatus sp. nov.
http://zoobank.org:8080/NomenclaturalActs/2332FBCD-A6F6-4583-AB61-D25F0D6B784C
(Figs. 8, 9)

Type material.—*Holotype juvenile.* AUSTRALIA: *Western Australia*: juvenile, Kens Bore, 49.7 km S. of Pannawonica, 22°05′11″S, 116°14′03″E, 7–10 July 2009, troglofauna trap, J. Cairnes, D. Keirle (Biota Environmental Sciences, KBRC039P8T2–5) (WAM T98698) (DNA: *COI*, ITS2).

Etymology.—The specific epithet refers to the discovery of this species only by molecular methods (*celatus*, Latin, hidden).

Diagnosis.—Adults of *P. celatus* are unknown, but specimens of this species can be distinguished from all other species of *Paradraculoides* by the following combination of nucleotide substitutions: *COI* ($n = 1$): 86 (G), 205 (T), 241 (C), 301 (T),

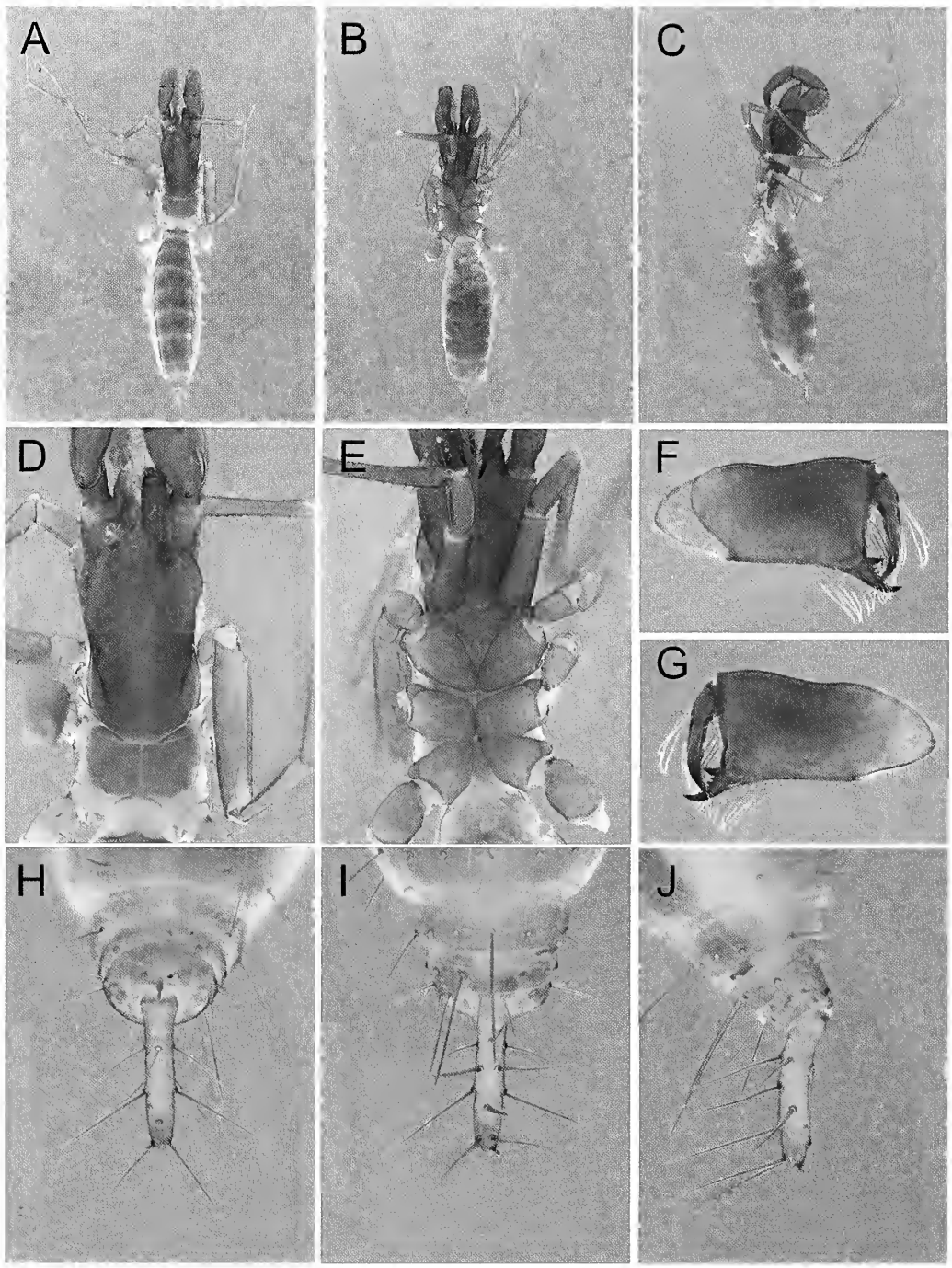

Figure 6.—*Paradraculoides catho* sp. nov., holotype female (WAM T93192): A. Body, dorsal; B. Body, ventral; C. Body, lateral; D. Cephalothorax, dorsal; E. Cephalothorax, ventral; F. Left chelicera, prolateral; G. Left chelicera, retrolateral. H. Flagellum, dorsal; I. Flagellum, ventral. Abbreviations: dm1, 4 (dorso-median 1, 4), dl1, 3 (dorso-lateral 1, 3), vm1, 2, 3, 5 (ventro-median 1, 2, 3, 5), vl1, 2 (ventro-lateral 1, 2).

370 (T), 388 (C), 625 (C), 799 (C); 12S ($n = 1$): 4 (T), 22 (A), 197 (A), 202 (G), 239 (G), 326 (A), 333 (A) (Table 2).

Description (juvenile).—*Color:* Pale yellow-brown (Figs. 8A–C).

Cephalothorax: Propeltidium with 2 apical setae followed by single seta on anterior process and 2 + 2 setae; eye spots absent. Mesopeltidia separated. Anterior sternum with 12 setae (including 2 sternapophysial setae); posterior sternum triangular with 6 setae.

Chelicera: Fixed finger with 2 large teeth plus 4 smaller teeth between these, plus 1 very small tooth on margin of large teeth; brush at base of fixed finger composed of 7 setae (G5A), each densely pilose in distal half; lateral surface with 3 large, lanceolate, terminally pilose setae (G1); internal face of chelicera with 5 short whip-like setae (G4); movable finger serrula composed of 14 long lamellae, blunt guard tooth present subdistally; 1 accessory tooth present at two-thirds from base of serrula.

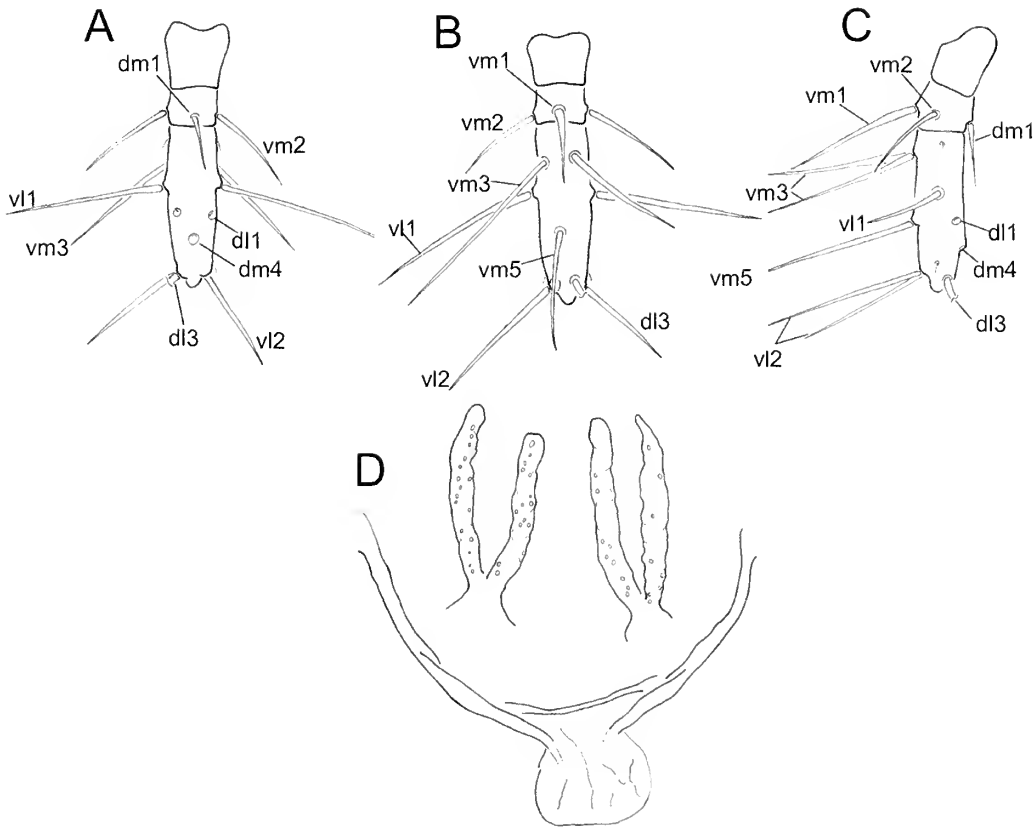

Figure 7.—*Paradraculoides catho* sp. nov., holotype female (WAM T93192): A. Flagellum, dorsal; B. Flagellum, ventral; C. Flagellum, lateral; D. Spermatheeae, ventral. Abbreviations: dm1, 4 (dorso-median 1, 4), dl1, 3 (dorso-lateral 1, 3), vm1, 2, 3, 5 (ventro-median 1, 2, 3, 5), vl1, 2 (ventro-lateral 1, 2).

Pedipalp: Without apophyses; trochanter with sharply produced ventro-distal extension, ventral margin with ca. 8 stout setae, without mesal spur; tarsus and tibia without spines; tarsal spur present; claw 0.50 x length of tarsus.

Legs: Tarsus I with 6 tarsomeres; femur IV 2.60 x longer than wide; baso-dorsal margin of femur IV produced at about a 90° angle.

Abdomen: Chaetotaxy of tergites I–IX: 2 macrosetae + 4 microsetae: 3 macrosetae + 6 microsetae (microsetae in column): 2: 2: 2: 2: 2: 2: 4.

Flagellum: 5.00 x longer than broad; seta dm1 situated towards posterior end of flagellomere II slightly more posterior than vm2; setae dl1 situated anterior to dm4, dm4 situated at about three-quarters the length on flagellomere III; dl3 situated near posterior margin, very slightly posterior to vl2; vm1 situated between vm2, slightly anterior to vm2; vm3 situated slightly closer to vm1 than to vm5; vm5 halfway between vm3 and vl2; vl1 situated posterior to vm3 and anterior to dl1; 1 pair of microsetae baso-laterally on flagellomere III, 1 pair of microsetae postero-laterally anterior to dl3.

Dimensions (mm): Holotype juvenile (WAM T98698): Body length 2.50. Propeltidium 0.82/0.44. Chelicera 0.51. Flagellum 0.25/0.05. Pedipalp: trochanter 0.31, femur 0.34, patella 0.38, tibia 0.33, tarsus 0.18, claw 0.09, total excluding claw 1.54. Leg I: trochanter 0.34, femur 0.78, patella 0.92, tibia 0.72, metatarsus 0.26, tarsus 0.46, total 3.48. Leg IV: trochanter 0.25, femur 0.78/0.30, patella 0.37, tibia 0.51, metatarsus 0.58, tarsus 0.39, total 2.88.

Remarks.—*Paradraculoides celatus* has only been collected from Kens Bore, Western Australia (Fig. 1) at a site that is only 4.7 km south-east of one of the two known localities of *P. obrutus* and 5.9 km north of *P. affinis*. The specimen was collected from a bore hole using troglofauna traps.

Paradraculoides cochranus sp. nov.
http://zoobank.org:8080/NomenclaturalActs/84eeea24-aad9-402e-b153-6836fbbcf59a
(Figs. 10–12)

Type material.—*Holotype male.* AUSTRALIA: *Western Australia*: Cochrane and Jewell, 37.7 km S. of Pannawonica, 21°55′55″S, 116°07′43″E, 25–28 November 2008, troglofauna trap, 15 m, J. Cairnes, M. Menz (Biota Environmental Sciences, RNRC083P5T1–3) (WAM T93229) (DNA: *COI*, ITS2).

Paratypes. AUSTRALIA: *Western Australia*: 1 ♀, Cochrane and Jewell, 37.7 km S. of Pannawonica, 21°55′55″S, 116°07′43″E, 13–16 October 2008, troglofauna trap, 15 m, G. Humphreys, M. Menz (Biota Environmental Sciences, RNRC083P4T1–3) (WAM T93197) (DNA: *COI*, ITS2); 1 ♀, Jewell and Cochrane Bore, site RNRC162, 21°56′02.88″S, 116°08′15.54″E, 17 January 2008, troglofauna trap, J. Alexander, T. Sachse (Biota Environmental Sciences, RNRC162P2T2–1) (WAM T54136).

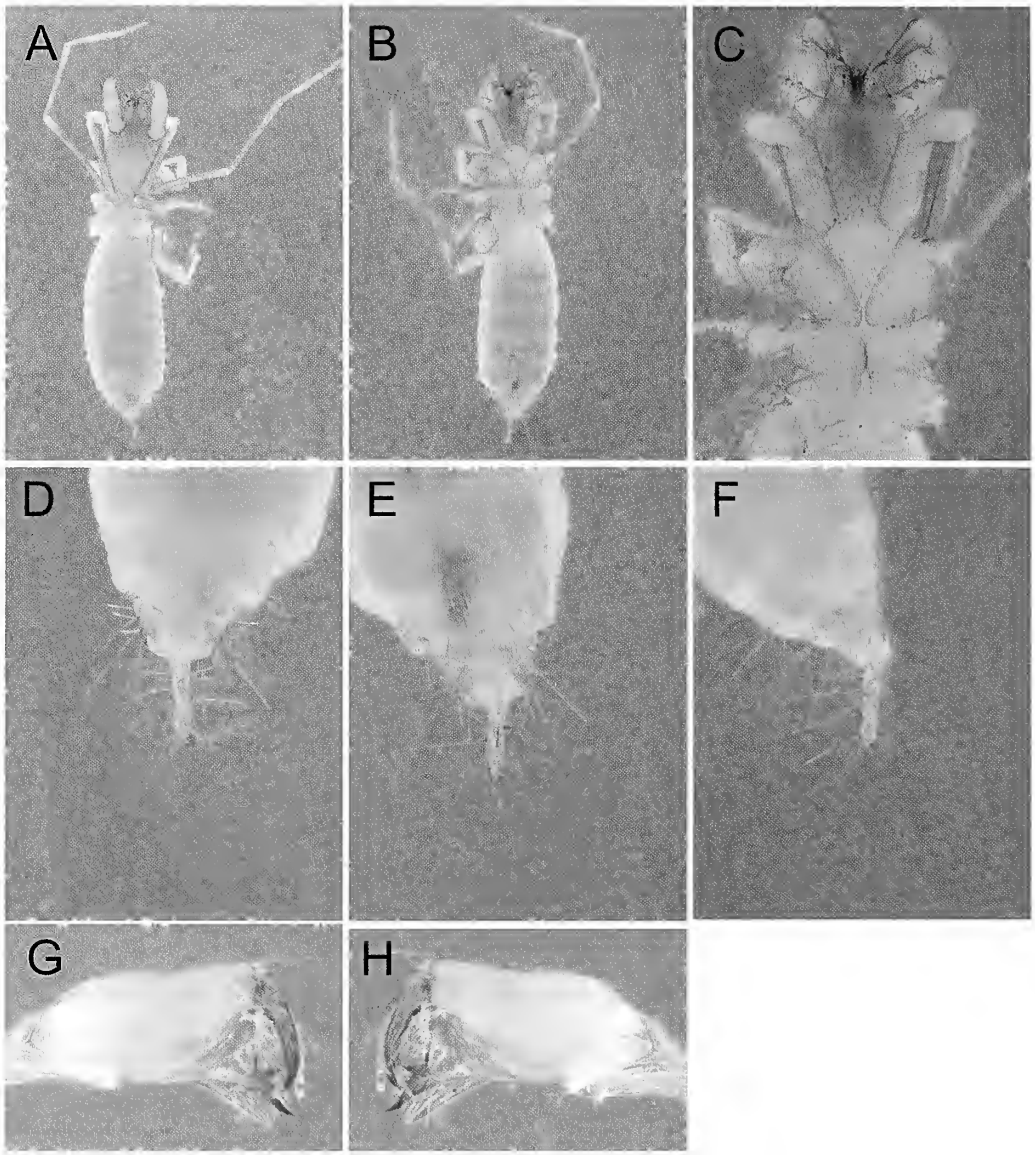

Figure 8.—*Paradraculoides celatus* sp. nov., holotype juvenile (WAM T98698): A. Body, dorsal; B. Body, ventral; C. Cephalothorax, ventral; D. Flagellum, dorsal; E. Flagellum, ventral; F. Flagellum, lateral; G. Left chelicera, prolateral; H. Left chelicera, retrolateral. Abbreviations: dm1, 4 (dorso-median 1, 4), dl1, 3 (dorso-lateral 1, 3), vm1, 2, 3, 5 (ventro-median 1, 2, 3, 5), vl1, 2 (ventro-lateral 1, 2).

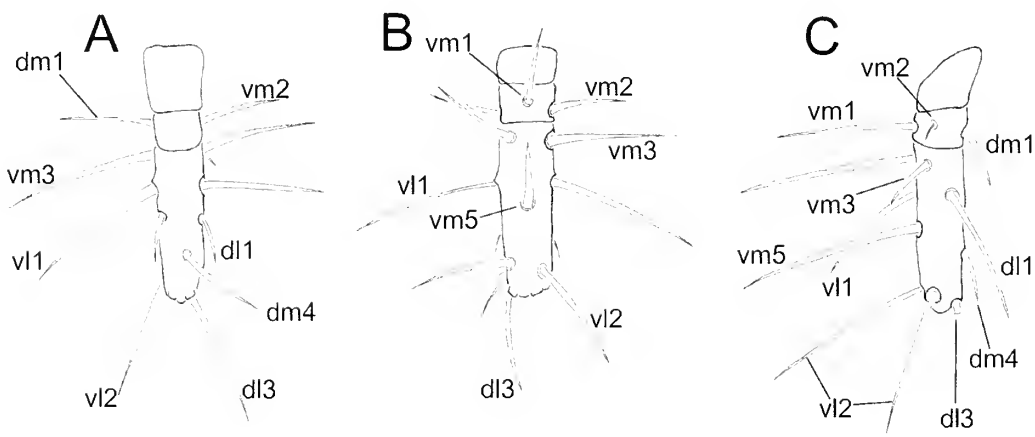

Figure 9.—*Paradraculoides celatus* sp. nov., holotype juvenile (WAM T98698): A. Flagellum, dorsal; B. Flagellum, ventral; C. Flagellum, lateral. Abbreviations: dm1, 4 (dorso-median 1, 4), dl1, 3 (dorso-lateral 1, 3), vm1, 2, 3, 5 (ventro-median 1, 2, 3, 5), vl1, 2 (ventro-lateral 1, 2).

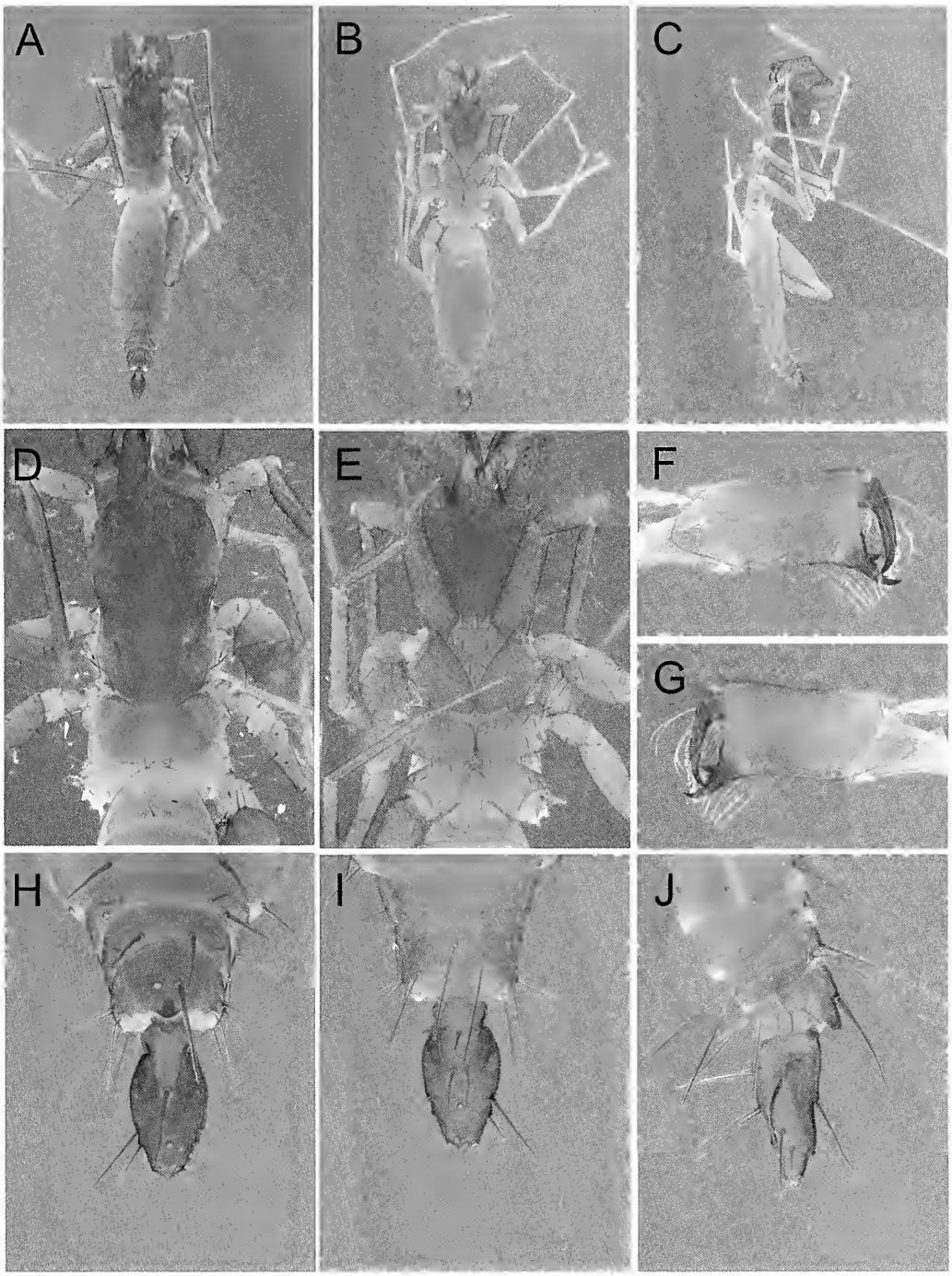

Figure 10.—*Paradraculoides cochranus* sp. nov., holotype male (WAM T93229): A. Body, dorsal; B. Body, ventral; C. Body, lateral; D. Cephalothorax, dorsal; E. Cephalothorax, ventral; F. Left chelicera, prolateral; G. Left chelicera, retrolateral; H. Flagellum, dorsal; I. Flagellum, ventral; J. Flagellum, lateral. Abbreviations: dm1, 4 (dorso-median 1, 4), dl1, 3 (dorso-lateral 1, 3), vm1, 2, 3, 5 (ventro-median 1, 2, 3, 5), vl1, 2 (ventro-lateral 1, 2).

Other material examined.—AUSTRALIA: *Western Australia*: 1 juvenile, Cochrane and Jewell, 37.7 km S. of Pannawonica, 21°55′55″S, 116°07′43″E (WAM T92538) (DNA: *COI*, ITS2); 1 ♂, Cochrane and Jewell, Yaraloola Station, NE. of Red Hill, 38.5 km S. of Pannawonica, 21°56′11″S, 116°07′14″E, 15–21 August 2008, troglofauna trap, 15 m, J. Alexander, T. Sachse (Biota Environmental Sciences, RNRC140P3T1–4) (WAM T92536); 1 ♀, Yaraloola Station, NE. of Red Hill, Cochrane and Jewell, 35.9 km S. of Pannawonica, 21°55′11″S, 116°08′35″E, 15–21 August 2008, troglofauna trap, 10 m, J. Alexander, T. Sachse (Biota Environmental Sciences, RNRC189P3T1–1) (WAM T92541); 1 ♂, Cochrane and Jewell, 35.5 km S. of Pannawonica, 21°55′09″S, 116°08′49″E, 25–28 November 2008, troglofauna trap, 30 m, J. Cairnes, M. Menz (Biota Environmental Sciences, RNRC184P5T3–1) (WAM T93232) (DNA: *COI*, ITS2); 1 ♀, Cochrane and Jewell, 35.5 km S. of Pannawonica, 21°55′09″S, 116°08′49″E, 25–28 November

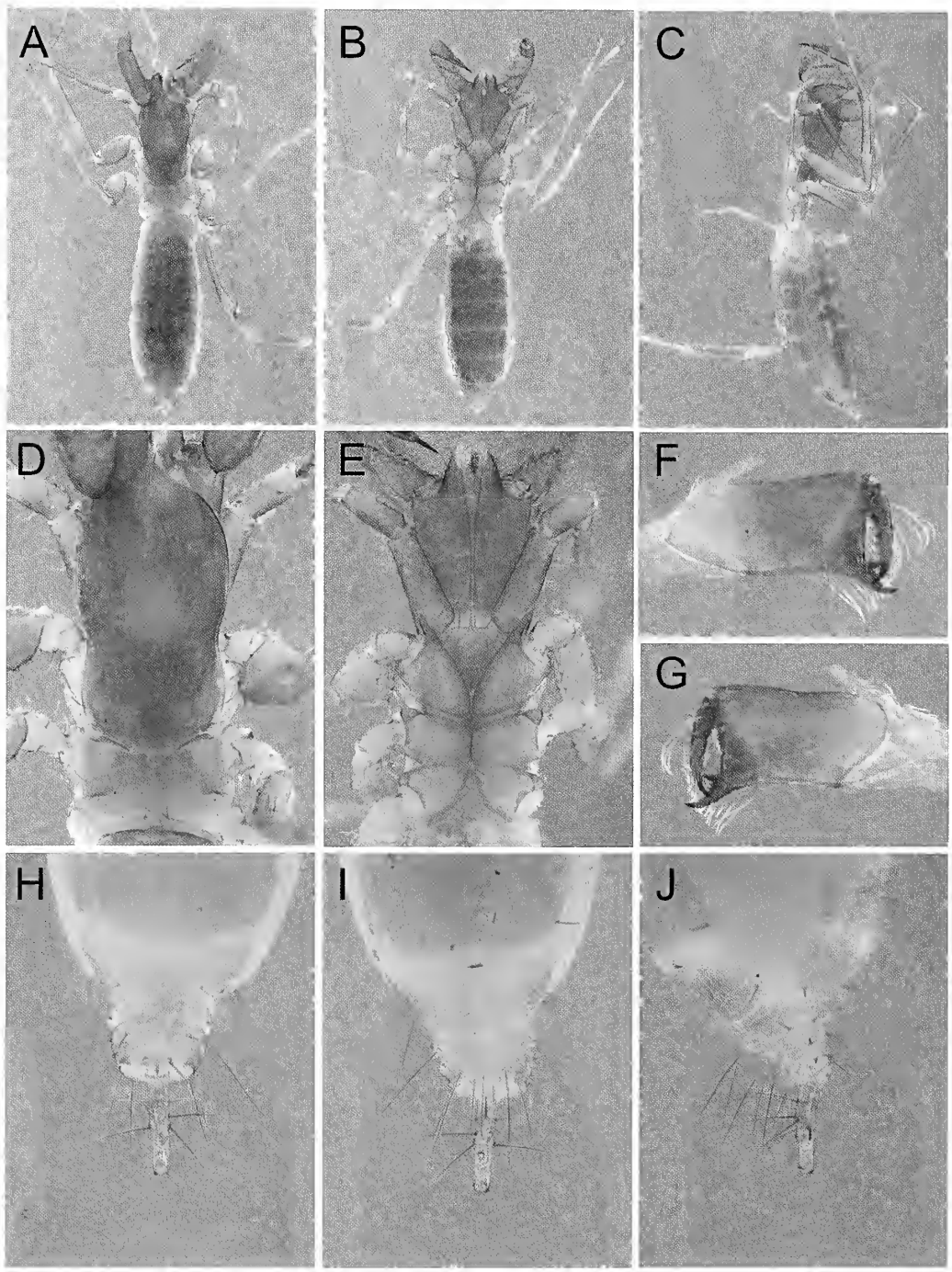

Figure 11.—*Paradraculoides cochranus* sp. nov., paratype female (WAM T93197): A. Body, dorsal; B. Body, ventral; C. Body, lateral; D. Cephalothorax, dorsal; E. Cephalothorax, ventral; F. Left chelicera, prolateral; G. Left chelicera, retrolateral; paratype female (WAM T54136): H. Flagellum, dorsal; I. Flagellum, ventral; J. Flagellum, lateral. Abbreviations: dm1, 4 (dorso-median 1, 4), dl1, 3 (dorso-lateral 1, 3), vm1, 2, 3, 5 (ventro-median 1, 2, 3, 5), vl1, 2 (ventro-lateral 1, 2).

2008, troglofauna trap, 10 m, J. Cairnes, M. Menz (Biota Environmental Sciences, RNRC184P5T1–1) (WAM T93230); 1 ♀, Cochrane and Jewell, 35.9 km S. of Pannawonica, 21°55′11″S, 116°08′35″E, 25–28 November 2008, troglofauna trap, 10 m, J. Cairnes, M. Menz (Biota Environmental Sciences, RNRC189P5T1–1) (WAM T93231) (DNA: *COI*, ITS2); 1 juvenile, Cochrane and Jewell, 35.5 km S. of Pannawonica, 21°55′09″S, 116°08′49″E, 13–16 October 2008, troglofauna trap, 20 m, G. Humphreys, M. Menz (Biota Environmental Sciences, RNRC184P4T2–3) (WAM T93191) (DNA: *COI*, ITS2); 1 juvenile, Jewell and Cochrane Bore, site RNRC048, 21°56′08.69″S, 116°08′52.71″E, 24 October 2007, troglofauna trap, D. Kamien, J. Alexander (Biota Environmental Sciences, RNRC048T3–2) (WAM T54129); 1 juvenile, Jewell and Cochrane Bore, site RNRC213, 21°54′42.64″S, 116°07′24.48″E, 24 October 2007, troglofauna trap, D.

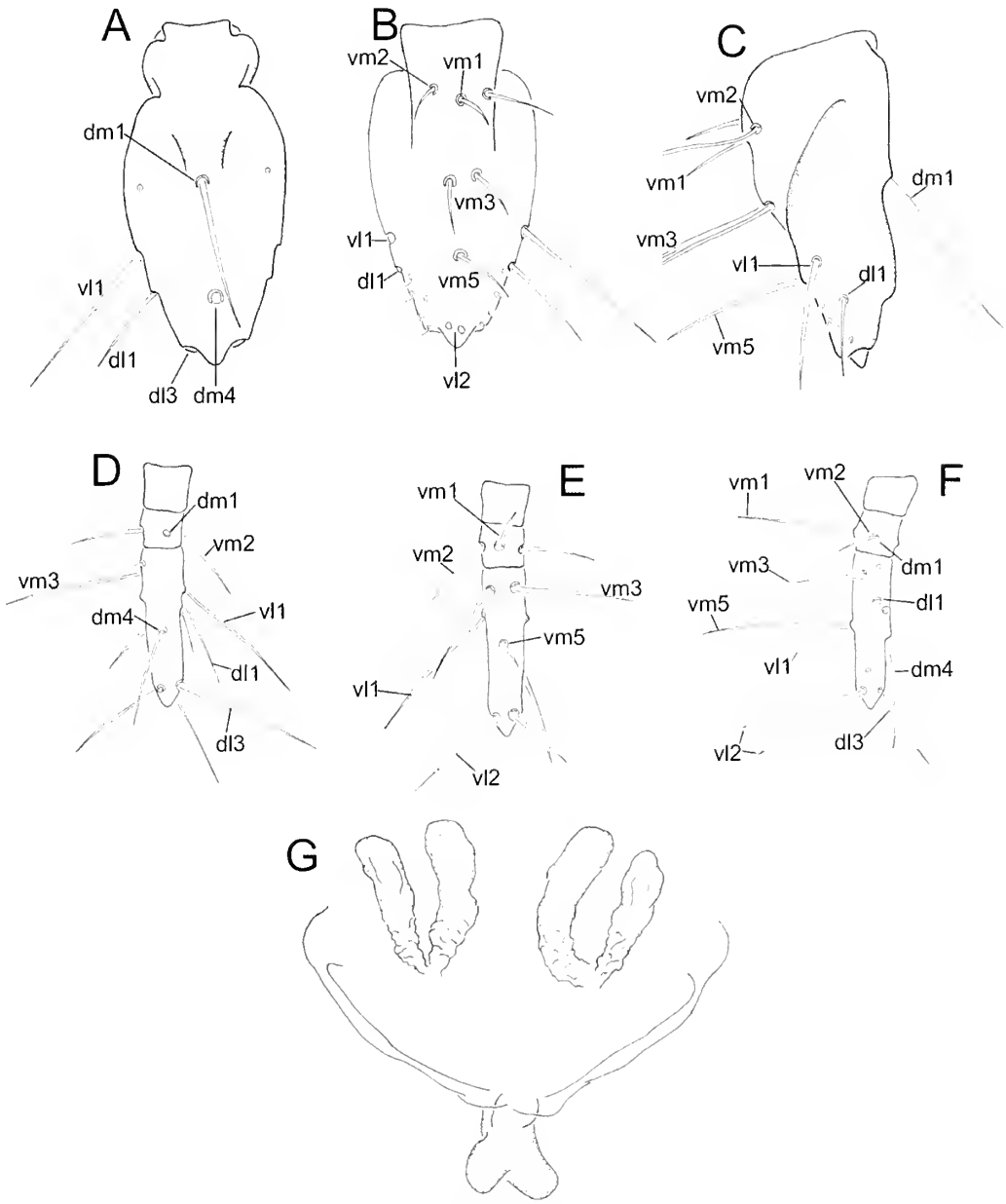

Figure 12.—*Paradraculoides cochranus* sp. nov.: A–C, holotype male (WAM T93229): A. Flagellum, dorsal; B. Flagellum, ventral; C. Flagellum, lateral. D–G, paratype female (WAM T93197): D. Flagellum, dorsal; E. Flagellum, ventral; F. Flagellum, lateral; G. Spermathecae, ventral. Abbreviations: dm1, 4 (dorso-median 1, 4), dl1, 3 (dorso-lateral 1, 3), vm1, 2, 3, 5 (ventro-median 1, 2, 3, 5), vl1, 2 (ventro-lateral 1, 2).

Kamien, J. Alexander (Biota Environmental Sciences, RNRC213T2–1) (WAM T54128); 1 juvenile, Jewell and Cochrane Bore, site RNRC094, 21°55′04.45″S, 116°08′17.95″E, 23 October 2007, troglofauna trap, D. Kamien, J. Alexander (Biota Environmental Sciences, RNRC094T3–3) (WAM T54134).

Etymology.—The specific epithet is an adjective that refers to the type locality, Cochrane and Jewell Bores.

Diagnosis.—The overall shape of the male flagellum of *P. cochranus* (Figs. 10H–J, 12A–C) most closely resembles *P. bythius*, *P. affinis*, *P. eremius* and *P. kryptus*, especially in the presence of a broad base, from which it differs by the position of dm4 which is located sub-distally (Fig. 12A) rather than on the posterior edge of the flagellum in the other species. The female flagellum of *P. cochranus* differs from all other *Paradraculoides* in the short gap between dm4 to dl1 (by the relatively anterior position of dm4) (Fig. 12D). Specimens of this species can be distinguished from all other species of *Paradraculoides* by the following combination of nucleotide substitutions: *COI* ($n = 6$): 133 (A), 235 (T), 263 (G), 279 (G) (Table 2).

Description (adults).—*Color:* Yellow-brown; propeltidium somewhat darker; female abdomen darker than male with olive-grey tinge (Figs. 10A–C, 11A–C).

Cephalothorax: Propeltidium with 2 apical setae followed by single seta on anterior process and 2 + 2 + 2 (male only) + 2 setae; eye spots absent. Mesopeltidia separated. Anterior

sternum with 12 (♂), 13 (♀) setae (including 2 sternapophysial setae); posterior sternum triangular with 6 setae.

Chelicera: Fixed finger with 2 large teeth plus 4 smaller teeth between these; distal surface of basal tooth with short blunt tooth (♂) or low protrusion (♀); brush at base of fixed finger composed of 10 (♂) or 7 (♀) setae (G5A), each densely pilose in distal half; lateral surface with 3 large, lanceolate, terminally pilose setae (G1); internal face of chelicera with 4 (♂, ♀) short whip-like setae (G4); movable finger serrula composed of 18 (♂), 17 (♀) long lamellae, blunt guard tooth present subdistally; 1 large accessory tooth present (♂, ♀) at two-thirds from base of serrula accompanied by smaller tooth basally (♀ only).

Pedipalp: Without apophyses; trochanter with sharply produced ventro-distal extension, ventral margin with ca. 10 stout setae, without mesal spur; tarsus and tibia without spines; tarsal spur present; claw 0.47 (♂), 0.44 (♀) x length of tarsus.

Legs: Tarsus I with 6 tarsomeres; femur IV 3.16 (♂), 2.85 (♀) x longer than wide; baso-dorsal margin of femur IV produced at about a 90° angle.

Abdomen: Chaetotaxy of tergites I–IX: 2 macrosetae + 4 microsetae: 3 macrosetae + 6 microsetae (microsetae in column): 2: 2: 2: 2: 2: 2: 4.

Female genitalia: Two pairs of spermathecae, each pair connected basally before connection with bursa (Fig. 12G), distally round and smooth; sparsely covered with small pores; gonopod short, distally bifurcate.

Flagellum: Male: dorsoventrally compressed (Figs. 10H–J, 12A–C); 2.00 x longer than broad; seta dm1 situated dorso-medially, slightly closer to anterior margin; seta dm4 situated at same level as dl1; dl3 on posterior margin; vm2 situated slightly anterior to vm1, vm1 much closer to vm2 than to vm3; vm5 situated halfway between vl2 and vm3; 1 pair of microsetae at similar level as dm1 and four pairs between vl1 and dl3. Female: 4.11 x longer than broad; seta dm1 situated towards posterior end of flagellomere II slightly more posterior than vm2; setae dl1 situated anterior to dm4, dm4 situated about halfway on flagellomere III; dl3 situated near at posterior margin at same level as vl2; vm1 situated between vm2; vm3 situated closer to vm1 than to vm5, vm5 halfway between vm3 and vl2; vl1 situated posterior to vm3 and anterior to dl1; 1 pair of microsetae baso-laterally on flagellomere III, 1 pair of microsetae postero-laterally but well anterior of dl3 and vl2.

Dimensions (mm): Holotype male (WAM T93229): Body length 4.33. Propeltidium 1.34/0.75. Chelicera 0.72. Flagellum 0.46/0.23. Pedipalp: trochanter 0.58, femur 0.67, patella 0.71, tibia 0.65, tarsus 0.33, claw 0.15, total excluding claw 2.94. Leg I: trochanter 0.44, femur 1.81, patella 2.32, tibia 1.84, metatarsus 0.52, tarsus 0.79, total 7.72. Leg IV: trochanter 0.46, femur 1.61/0.27, patella 0.61, tibia 1.23, metatarsus 1.09, tarsus 0.61, total 5.63.

Paratype female (WAM T93197): Body length 4.34. Propeltidium 1.34/0.67. Chelicera 0.72. Flagellum 0.37/0.09. Pedipalp: trochanter 0.58, femur 0.61, patella 0.67, tibia 0.60, tarsus 0.31, claw 0.13, total excluding claw 2.77. Leg I: trochanter 0.40, femur 1.40, patella 1.77, tibia 1.40, metatarsus 0.40, tarsus 0.69, total 6.06. Leg IV: trochanter 0.38, femur 1.33/0.19, patella 0.54, tibia 0.96, metatarsus 0.83, tarsus 0.54, total 4.58.

Variation: Body length (males, $n = 3$) 4.08–4.33, (females, $n = 5$) 3.15–4.34; propeltidium (males) 1.11–1.34/0.56–0.75, (females) 1.11–1.34/0.52–0.71.

Remarks.—*Paradraculoides cochranus* is known from the Cochrane and Jewell Bores complex, located in the western Hamersley Range, Western Australia, midway between the distributions of *P. celatus* and *P. gnophicola* (Fig. 1). The juvenile specimens listed above are associated with this species by locality and, in some cases, by sequence data (Figs. 2A–D).

Paradraculoides confusus sp. nov.
http://zoobank.org:8080/NomenclaturalActs/03B68E37-083A-4A41-9C76-BDDC2715D1A1
(Figs. 13, 14)

Type material.—*Holotype male.* AUSTRALIA: *Western Australia*: Trinity Bore, 76.4 km S. of Pannawonica, 22°19′57″S, 116°21′32″E, 15–21 August 2008, troglofauna trap, 25 m, J. Alexander, T. Sachse (Biota Environmental Sciences, TBRC014T1) (WAM T93142) (DNA: *COI*, ITS2).

Etymology.—The specific epithet is the Latin adjective for confused (*confusus*), as this species shares both the diagnostic characters of *Draculoides* (laterally compressed male flagellum) and the former diagnostic character of *Paradraculoides* (three setae on tergite II).

Diagnosis.—The male of *P. confusus* differ from all other species of the genus by the laterally compressed flagellum (Figs. 13H–J, 14A–C). Females are unknown. Specimens of this species can also be distinguished from all other species of *Paradraculoides* by the following combination of nucleotide substitutions: *COI* ($n = 1$): 325 (C), 518 (G), 535 (C), 565 (C), 574 (C); ITS2 ($n = 1$): 251 (T), 271 (T); 28S ($n = 1$): 940 (A) (Table 2).

Description (adult male).—*Color:* Yellow-brown; propeltidium and 3–4 posterior abdominal sclerites somewhat darker (Figs. 13A–C).

Cephalothorax: Propeltidium with 2 + 1 apical setae on anterior process and 2 + 1 (left) + 2 setae; eye spots absent. Mesopeltidia separated. Anterior sternum with 13 setae (including 2 sternapophysial setae); posterior sternum triangular with 6 setae.

Chelicera: Fixed finger with two large teeth plus four smaller teeth between these; brush at base of fixed finger composed of 7 setae (G5A), each densely pilose in distal half; lateral surface with 3 large, lanceolate, terminally pilose setae (G1); internal face of chelicera with 5 short whip-like setae (G4); movable finger serrula composed of 16 long lamellae, blunt guard tooth present subdistally; one accessory tooth present at two-thirds from base of serrula.

Pedipalp: Without apophyses; trochanter with sharply produced ventro-distal extension, ventral margin with ca. 10 stout setae, without mesal spur; tarsus and tibia without spines; tarsal spur present; claw 0.50 length of tarsus.

Legs: Tarsus I with 6 tarsomeres; femur IV 4.63 x longer than wide; baso-dorsal margin of femur IV produced at about a 90° angle.

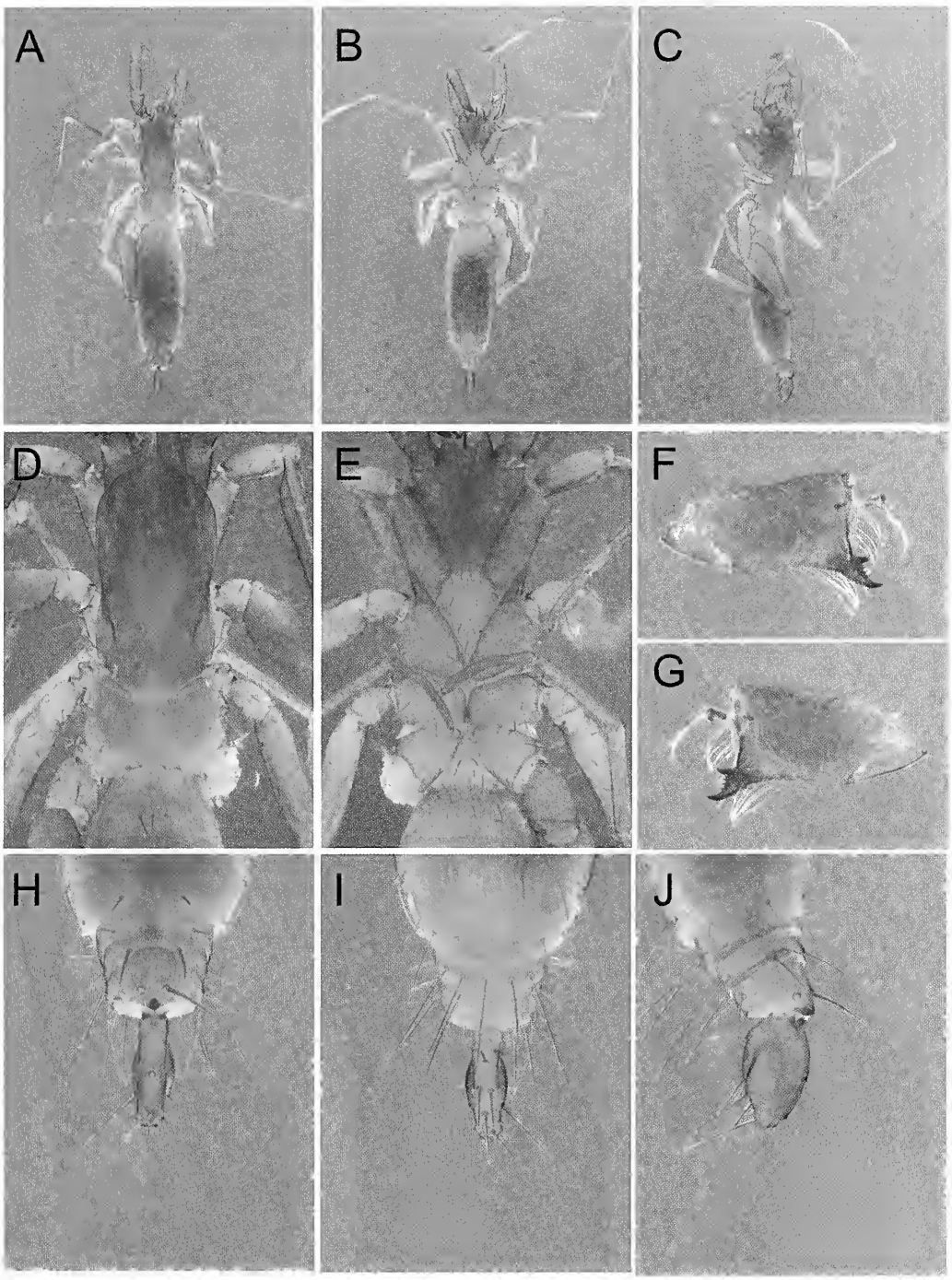

Figure 13.—*Paradraculoides confusus* sp. nov., holotype male (WAM T93142): A. Body, dorsal; B. Body, ventral; C. Body, lateral; D. Cephalothorax, dorsal; E. Cephalothorax, ventral; F. Left chelicera, prolateral; G. Left chelicera, retrolateral; H. Flagellum, dorsal; I. Flagellum, ventral; J. Flagellum, lateral. Abbreviations: dm1, 4 (dorso-median 1, 4), dl1, 3 (dorso-lateral 1, 3), vm1, 2, 3, 5 (ventro-median 1, 2, 3, 5), vl1, 2 (ventro-lateral 1, 2).

Abdomen: Chaetotaxy of tergites I–IX: 2 macrosetae + 1 microseta: 3 macrosetae (central setae half the size of laterals) + 6 microsetae (microsetae in column): 2: 2: 2: 2: 2: 2: 4.

Flagellum: Laterally compressed (Figs. 13A–C, 14A–C); 2.75 x longer than broad; seta dm1 situated dorso-medially, somewhat closer to anterior than to posterior margin; seta dm4 closer to posterior margin than to dm1; dl1 between dm4 and vl2; dl3 on posterior margin at similar level as dl1; vm2 situated anterior to vm1, vm1 closer to vm2 than to vm3; vm5 situated midway between vm3 and vl2; 1 pair of microsetae near anterior end, one pair centrally slightly above midline and three further pairs between dl1, vl1 and vl2.

Dimensions (mm): Holotype male (WAM T93142): Body length 3.74. Propeltidium 1.02/0.40. Chelicera 0.62. Flagellum 0.33/0.12. Pedipalp: trochanter 0.37, femur 0.44, patella 0.46, tibia 0.42, tarsus 0.23, claw 0.12, total excluding claw 1.92. Leg

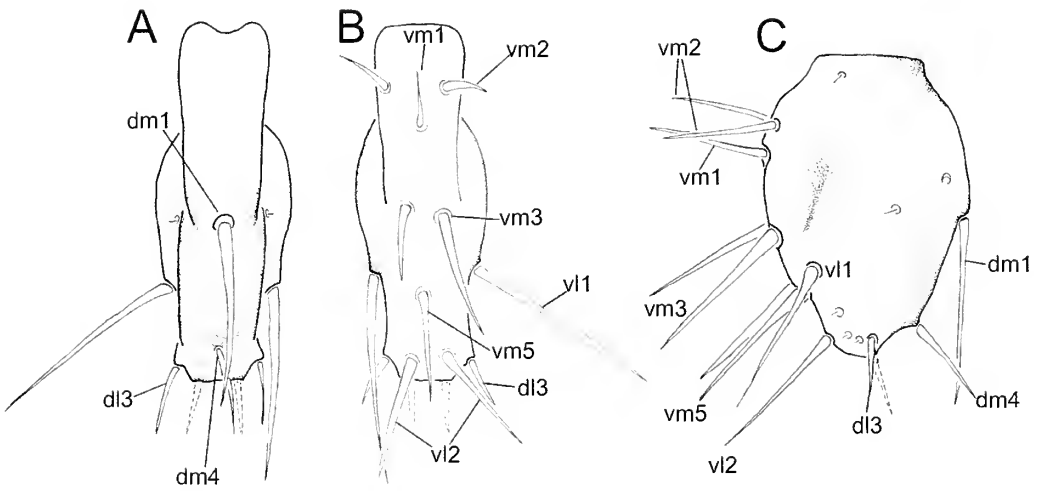

Figure 14.—*Paradraculoides confusus* sp. nov., holotype male (WAM T93142): A. Flagellum, dorsal; B. Flagellum, ventral; C. Flagellum, lateral. Abbreviations: dm1, 4 (dorso-median 1, 4), dl1, 3 (dorso-lateral 1, 3), vm1, 2, 3, 5 (ventro-median 1, 2, 3, 5), vl1, 2 (ventro-lateral 1, 2).

I: trochanter 0.31, femur 1.15, patella 1.38, tibia 1.13, metatarsus 0.37, tarsus 0.60, total 4.94. Leg IV: trochanter 0.29, femur 1.11/0.24, patella 0.46, tibia 0.81, metatarsus 0.71, tarsus 0.50, total 3.88.

Remarks.—*Paradraculoides confusus* has the primary diagnostic feature of *Paradraculoides*, three setae on tergite II, and the diagnostic feature of *Draculoides*, the laterally compressed male flagellum (Figs. 13A–C, 14A–C), suggesting an intermediate taxonomic position that questions the current hypothesis of two separate genera. A similar result was found by Clouse et al. (2017) who found that two species of *Paradraculoides* rendered *Draculoides* as paraphyletic. This taxonomic issue will be addressed in a forthcoming review of the phylogeny and taxonomy of the Pilbara schizomids (K. Abrams, J. Huey, M. Harvey, unpublished data).

Paradraculoides confusus is known from Trinity Bore, Western Australia, in the western Hamersley Range, where it occurs in a single mesa formation (Fig. 1). The mesa is adjacent to an extensive linear mesa, in which *P. trinity* occurs, but the mesas are separated by a low valley containing an incised drainage feature, suggesting that gene flow has been historically disrupted by erosion and separation of the Channel Iron Deposit habitat.

Paradraculoides obrutus sp. nov.
http://zoobank.org:8080/NomenclaturalActs/388D323E-E217-4DDC-81C6-6D4B0DCA6B08
(Figs. 15, 16)

Type material.—*Holotype female.* AUSTRALIA: *Western Australia*: Kens Bore, site KBRC023, 22°03′42.7″S, 116°11′44.29″E, 17 January 2008, troglofauna trap, J. Alexander, T. Sachse (Biota Environmental Sciences, KBRC023P2T2–1) (WAM T54175) (DNA: *COI*, ITS2).

Paratype. AUSTRALIA: *Western Australia*: 1 ♀, Kens Bore, 47.2 km S. of Pannawonica, 22°02′43″S, 116°11′03″E, 12–15 September 2009, troglofauna trap, 20 m, D. Kamien (Biota Environmental Sciences, KBRC096P7T2–1) (WAM T98320) (DNA: *COI*, ITS2).

Etymology.—The specific epithet refers to the discovery of this species only by molecular methods (*obrutus*, Latin, buried, hidden).

Diagnosis.—Males are unknown, and females cannot be differentiated from other species of *Paradraculoides* using morphological criteria. Specimens of this species can be distinguished from all other species of *Paradraculoides* by the following combination of nucleotide substitutions: *COI* ($n = 2$): 85 (T), 154 (T), 187 (C), 274 (C), 310 (T), 485 (T), 562 (C), 631 (T), 722 (G), 724 (A), 764 (T), 853 (C); ITS2 ($n = 2$): 199 (G), 281 (C); 28S ($n = 2$): 755 (A) (Table 2).

Description (adult female).—*Color:* Yellow-brown, chelicerae, pedipalps and anterior portion of propeltidium slightly darker (Figs. 15A–C).

Cephalothorax: Propeltidium with 2 apical setae followed by single seta on anterior process and 2 + 2 + 2 setae; eye spots absent. Mesopeltidia separated. Anterior sternum with 14 setae (including 2 sternapophysial setae); posterior sternum triangular with 7 setae.

Chelicera: Fixed finger with 2 large teeth plus 5 smaller teeth between these; distal surface of basal tooth with short blunt tooth; brush at base of fixed finger composed of 7 setae (G5A), each densely pilose in distal half; lateral surface with 3 large, lanceolate, terminally pilose setae (G1); internal face of chelicera with 4 short whip-like setae (G4); movable finger serrula composed of 16 long lamellae, blunt guard tooth present subdistally; 1 large accessory tooth present at two-thirds from base of serrula.

Pedipalp: Without apophyses; trochanter with sharply produced ventro-distal extension, ventral margin with ca. 8 stout setae, without mesal spur; tarsus and tibia without spines; tarsal spur present; claw 0.48 x length of tarsus.

Legs: Tarsus I with 6 tarsomeres; femur IV 2.60 x longer than wide; baso-dorsal margin of femur IV produced at about a 90° angle.

Abdomen: Chaetotaxy of tergites I–IX: 2 macrosetae + 4 microsetae: 3 macrosetae + 6 microsetae (microsetae in column): 2: 2: 2: 2: 2: 4: 6.

Female genitalia: Two pairs of spermathecae, each pair connected basally before connection with bursa (Fig. 16D),

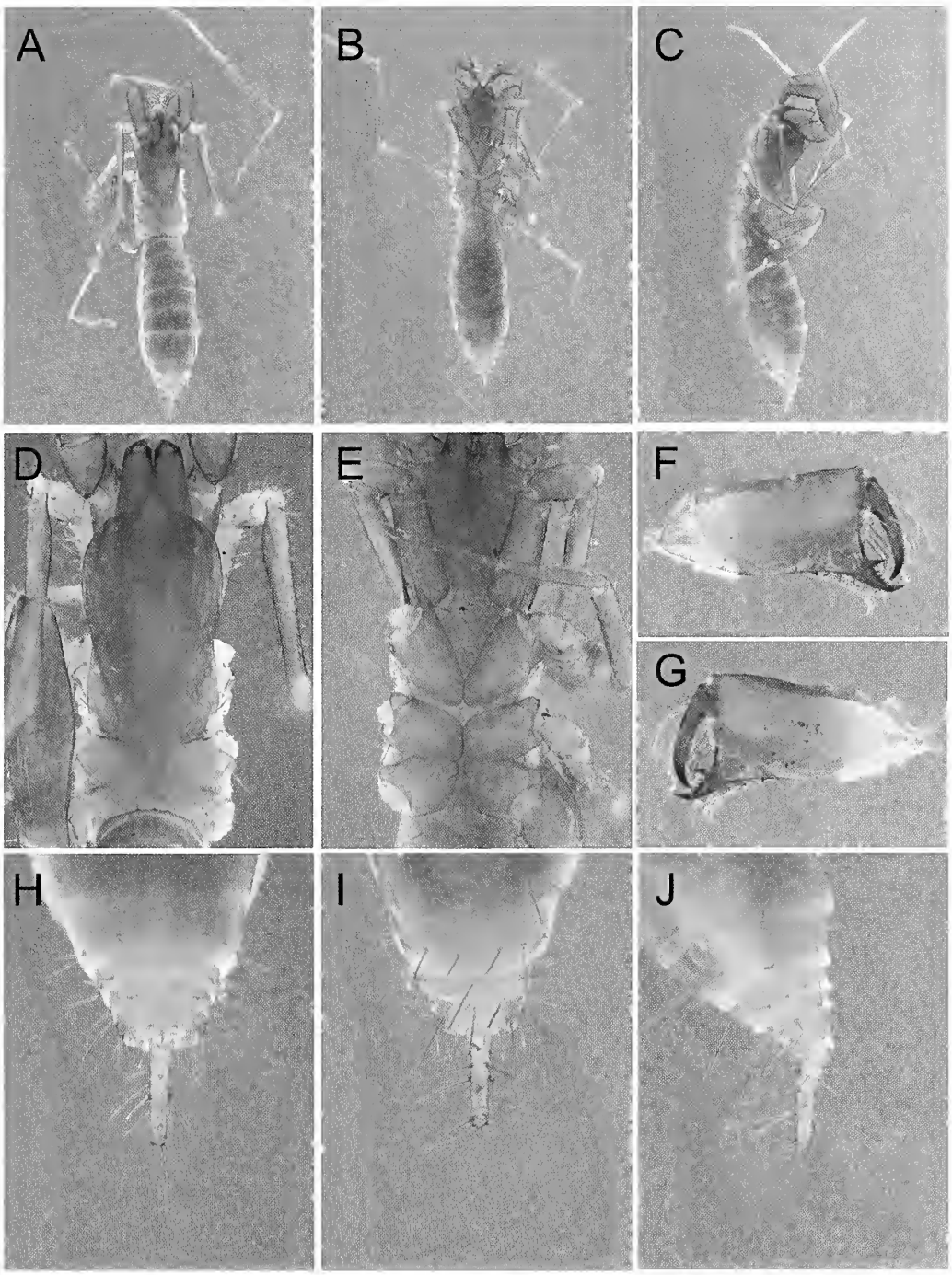

Figure 15.—*Paradraculoides obrutus* sp. nov., holotype female (WAM T54175): A. Body, dorsal; B. Body, ventral; C. Body, lateral; D. Cephalothorax, dorsal; E. Cephalothorax, ventral; F. Left chelicera, prolateral; G. Left chelicera, retrolateral; H. Flagellum, dorsal; I. Flagellum, ventral; J. Flagellum, lateral. Abbreviations: dm1, 4 (dorso-median 1, 4), dl1, 3 (dorso-lateral 1, 3), vm1, 2, 3, 5 (ventro-median 1, 2, 3, 5), vl1, 2 (ventro-lateral 1, 2).

distally round and smooth; sparsely covered with small pores; gonopod short, distally slightly bifureate.

Flagellum: 4.25 x longer than broad (Fig. 16D); seta dm1 situated in middle of flagellomere II slightly more posterior than vm2; setae dl1 situated anterior to dm4, dm4 situated at about three-quarters the length on flagellomere III; dl3 situated near posterior margin; vl2 situated away from posterior margin; vm1 situated between vm2; vm3 situated about halfway between vm1 and vm5; vm5 about halfway between vm3 and vl2; vl1 situated posterior to vm3 and anterior to dl1; 1 pair of microsetae postero-laterally anterior of dl3 and vl2.

Dimensions (mm): Holotype female (WAM T54175): Body length 3.35. Propeltidium 1.04/0.55. Chelicera 0.76. Flagellum 0.34/0.08. Pedipalp: trochanter 0.47, femur 0.46, patella 0.50, tibia 0.45, tarsus 0.27, claw 0.13, total exeluding claw 2.15. Leg I: trochanter 0.34, femur 1.13, patella 1.36, tibia 1.04, metatarsus 0.35, tarsus 0.53, total 4.07. Leg IV: trochanter

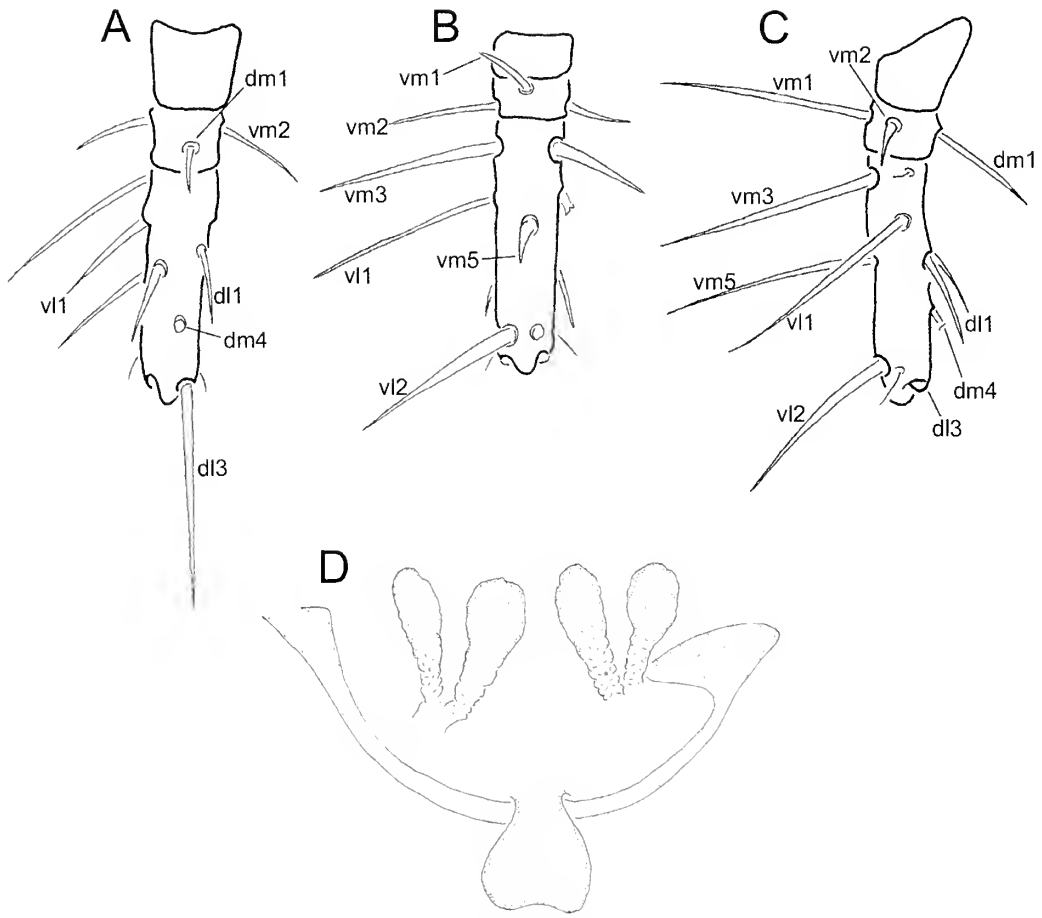

Figure 16.—*Paradraculoides obrutus* sp. nov., holotype female (WAM T54175): A. Flagellum, dorsal; B. Flagellum, ventral; C. Flagellum, lateral; D. Spermathecae, ventral. Abbreviations: dm1, 4 (dorso-median 1, 4), dl1, 3 (dorso-lateral 1, 3), vm1, 2, 3, 5 (ventro-median 1, 2, 3, 5), vl1, 2 (ventro-lateral 1, 2).

0.33, femur 1.04/0.40, patella 0.48, tibia 0.79, metatarsus 0.72, tarsus 0.51, total 3.93.

Variation: Body length (female, $n = 1$) 3.60; propeltidium 1.11/0.59.

Remarks.—This species is only known from two localities at Kens Bore, Western Australia (Fig. 1). The specimens were associated by the sequence data (Figs. 2A–D).

Paradraculoides trinity sp. nov.
http://zoobank.org:8080/NomenclaturalActs/D4DE9D6E-BC9C-44EA-850C-7E07DB3EC381
(Figs. 17–19)

Type material.—*Holotype male.* AUSTRALIA: *Western Australia*: Trinity Bore, 79.6 km S. of Pannawonica, 22°21′45″S, 116°19′13″E, 15–21 August 2008, troglofauna trap, 30 m, J. Alexander, T. Saehse (Biota Environmental Sciences, TBRC036P3T3–4) (WAM T92510) (DNA: *COI*, ITS2).

Paratypes. AUSTRALIA: *Western Australia*: 1 ♂, Trinity Bore, 79 km S. of Pannawonica, 22°21′35″S, 116°19′54″E, 15–21 August 2008, troglofauna trap, 45 m, J. Alexander, T. Saehse (Biota Environmental Sciences, TBRC023P3T3–1) (WAM T92511) (DNA: *COI*, ITS2); 1 ♀, Trinity Bore, 79 km S. of Pannawonica, 22°21′35″S, 116°19′54″E, 15–21 August 2008, troglofauna trap, 15 m, J. Alexander, T. Saehse (Biota Environmental Sciences, TBRC023P3T1–1) (WAM T92515) (DNA: *COI*); 1 ♀, Trinity Bore, 77.8 km S. of Pannawonica, 22°20′59″S, 116°20′17″E, 7–10 July 2009, troglofauna trap, 20 m, J. Cairnes, D. Keirle (Biota Environmental Sciences, TBRC161P8T2–4) (WAM T98704) (DNA: *COI*).

Other material examined.—AUSTRALIA: *Western Australia*: 1 juvenile, Trinity Bore, 79 km S. of Pannawonica, 22°21′35″S, 116°19′54″E, 15–21 August 2008, troglofauna trap, 45 m, J. Alexander, T. Saehse (Biota Environmental Sciences, TBRC023P3T3–1) (WAM T143827).

Etymology.—The specific epithet refers to the type locality, Trinity Bore, and is to be treated as a noun in apposition.

Diagnosis.—*Paradraculoides trinity* differs from all other species of *Paradraculoides* by the distinct shape of the male flagellum (Figs. 17H–J, 19A–C); in dorsal view the base is widest rendering the shape like the blunt tip of an arrow. The female flagellum and internal genitalia can currently not be distinguished with certainty from that of *P. catho* (but see respective diagnoses for all other species). Specimens of this species can be distinguished from all other species of *Paradraculoides* by the following combination of nucleotide substitutions: *COI* ($n = 4$): 292 (T), 389 (A), 474 (T), 785 (T);

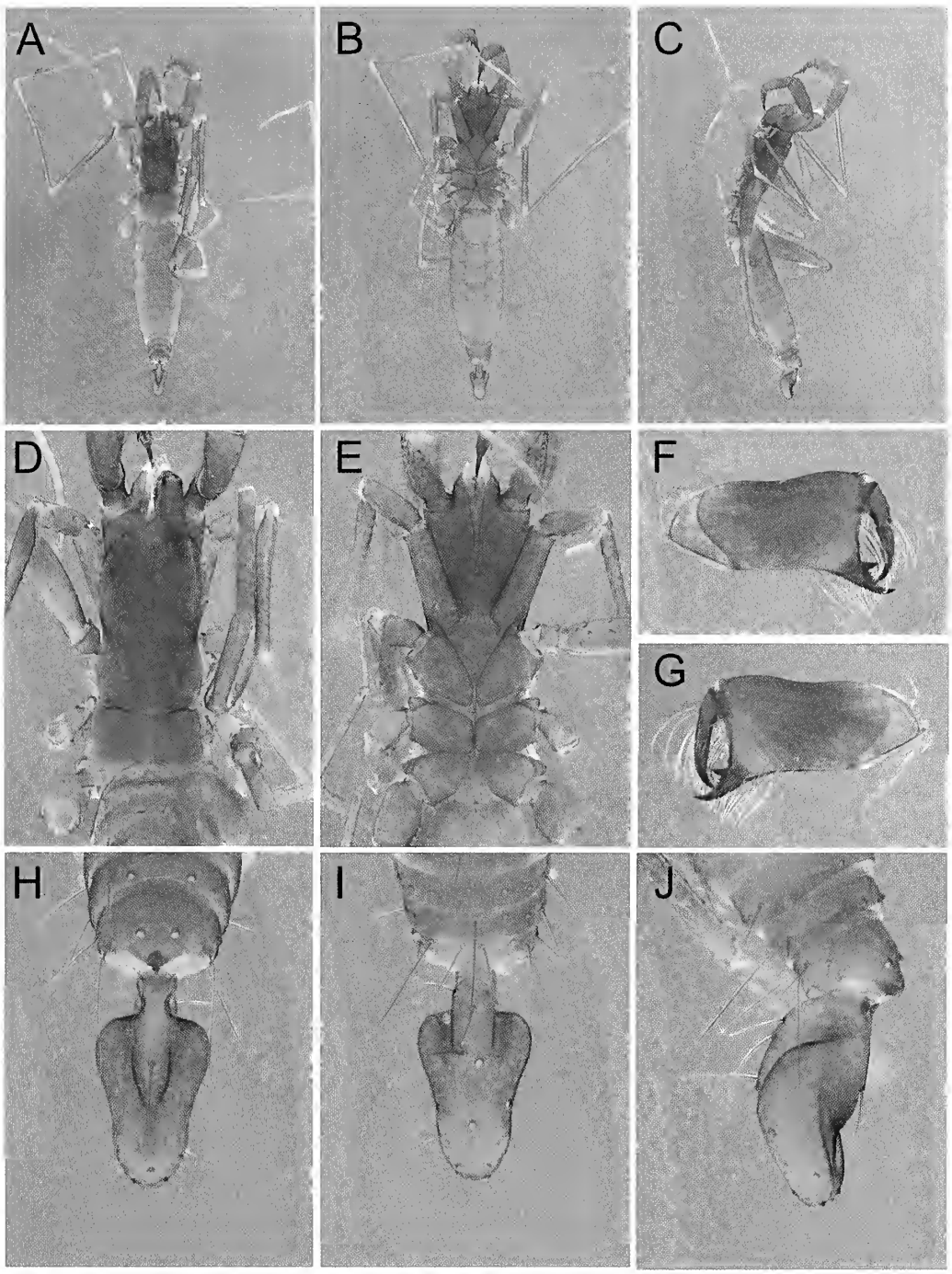

Figure 17.—*Paradraculoides trinity* sp. nov., holotype male (WAM T92510): A. Body, dorsal; B. Body, ventral; C. Body, lateral; D. Cephalothorax, dorsal; E. Cephalothorax, ventral; F. Left chelicera, prolateral; G. Left chelicera, retrolateral; H. Flagellum, dorsal; I. Flagellum, ventral; J. Flagellum, lateral. Abbreviations: dm1, 4 (dorso-median 1, 4), dl1, 3 (dorso-lateral 1, 3), vm1, 2, 3, 5 (ventro-median 1, 2, 3, 5), vl1, 2 (ventro-lateral 1, 2).

ITS2 ($n = 2$): 192 (A), 277 (G); 28S ($n = 1$): 469 (T), 647 (T), 661 (G) (Table 2).

Description (adults).—*Color:* Yellow-brown; propeltidium and pedipalps somewhat darker (Figs. 17A–C, 18A–C).

Cephalothorax: Propeltidium with 2 + 1 apical setae on anterior process and 2 + 2 + 2 setae; eye spots absent. Mesopeltidia separated. Anterior sternum with 12–13 (♂), 15 (♀) setae (including 2 sternapophysial setae); posterior sternum triangular with 6 or 8 (♂), 7 (♀) setae.

Chelicera: Fixed finger with 2 large teeth plus 5 smaller teeth between these, basal tooth with 1 lateral tooth; brush at base of fixed finger composed of 8 (♂), 10 (♀) setae (G5A), each densely pilose in distal half; lateral surface with 3 large, lanceolate, terminally pilose setae (G1); internal face of chelicera 5 short whip-like setae (G4); movable finger serrula

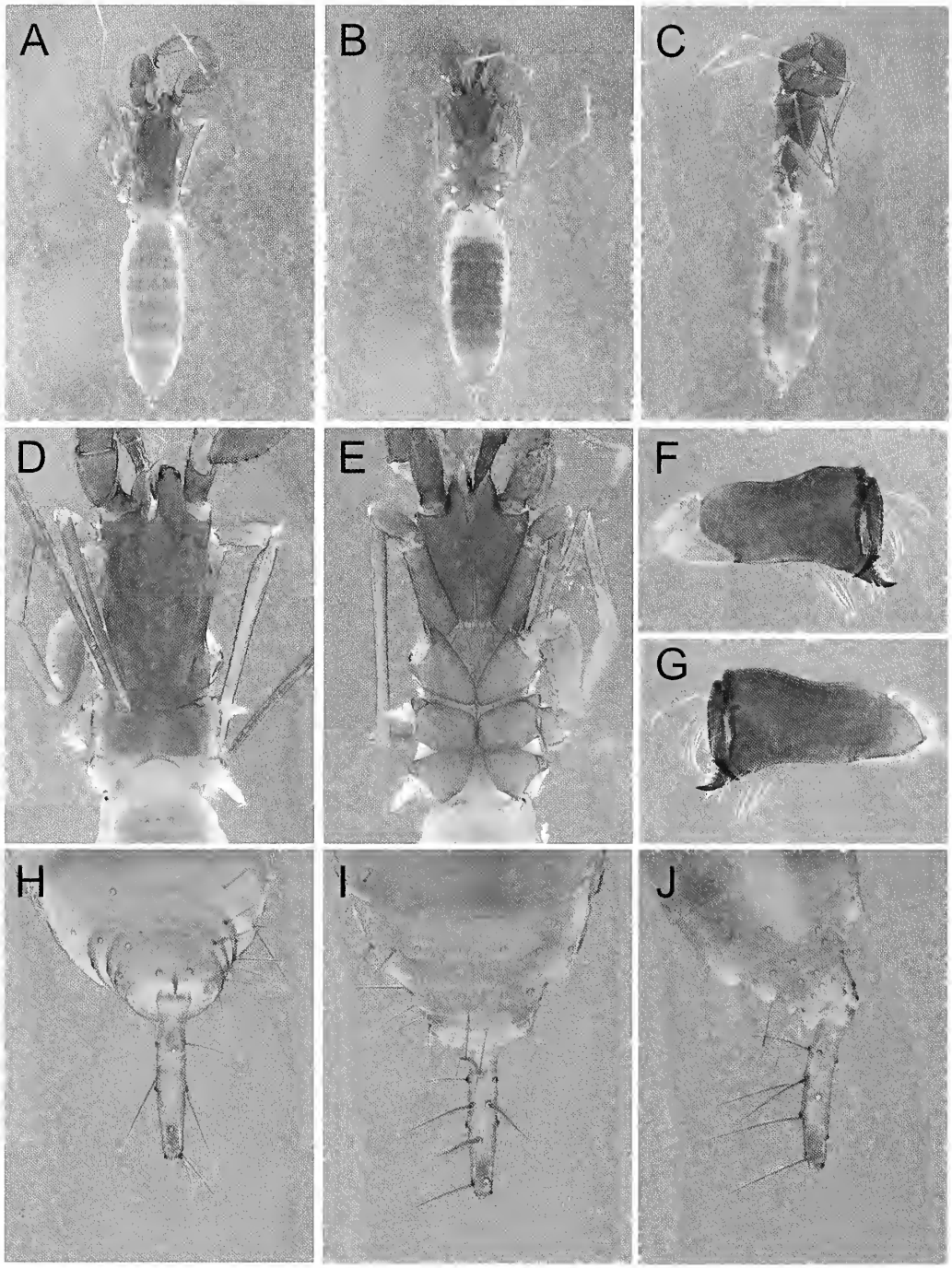

Figure 18.—*Paradraculoides trinity* sp. nov.: A–G, paratype female (WAM T92515): A. Body, dorsal; B. Body, ventral; C. Body, lateral; D. Cephalothorax, dorsal; E. Cephalothorax, ventral; F. Left chelicera, prolateral; G. Left chelicera, retrolateral; H–J, paratype female (WAM T98704): H. Flagellum, dorsal; I. Flagellum, ventral; J. Flagellum, lateral. Abbreviations: dm1, 4 (dorso-median 1, 4), dl1, 3 (dorso-lateral 1, 3), vm1, 2, 3, 5 (ventro-median 1, 2, 3, 5), vl1, 2 (ventro-lateral 1, 2).

composed of 17 (♂), 20 (♀) long lamellae, blunt guard tooth present subdistally; 2 accessory teeth present at two-thirds from base of serrula; the apical larger (in ♂ with two blunt tips).

Pedipalp: Without apophyses; troehanter with sharply produeed ventro-distal extension, ventral margin with ca. 15 stout setae, without mesal spur; tarsus and tibia without spines; tarsal spur present; claw 0.53 (♂), 0.43 (♀) x length of tarsus.

Legs: Tarsus I with 6 tarsomeres; femur IV 6.34 (♂), 5.97 (♀) x longer than wide; baso-dorsal margin of femur IV produeed at about a 90° angle.

Abdomen: Chaetotaxy of tergites I–IX: 2 macrosetae + 2 mierosetae: 3 macrosetae + 4 microsetae (microsetae in eolumn): 2: 2: 2: 2: 2: 2: 4.

Flagellum: Male: Dorsoventrally eompressed (Figs. 17H–J, 19A–C); 1.76 x longer than broad; seta dm1 situated at about 1 third of length of flagellum in anterior half; seta dm4 very

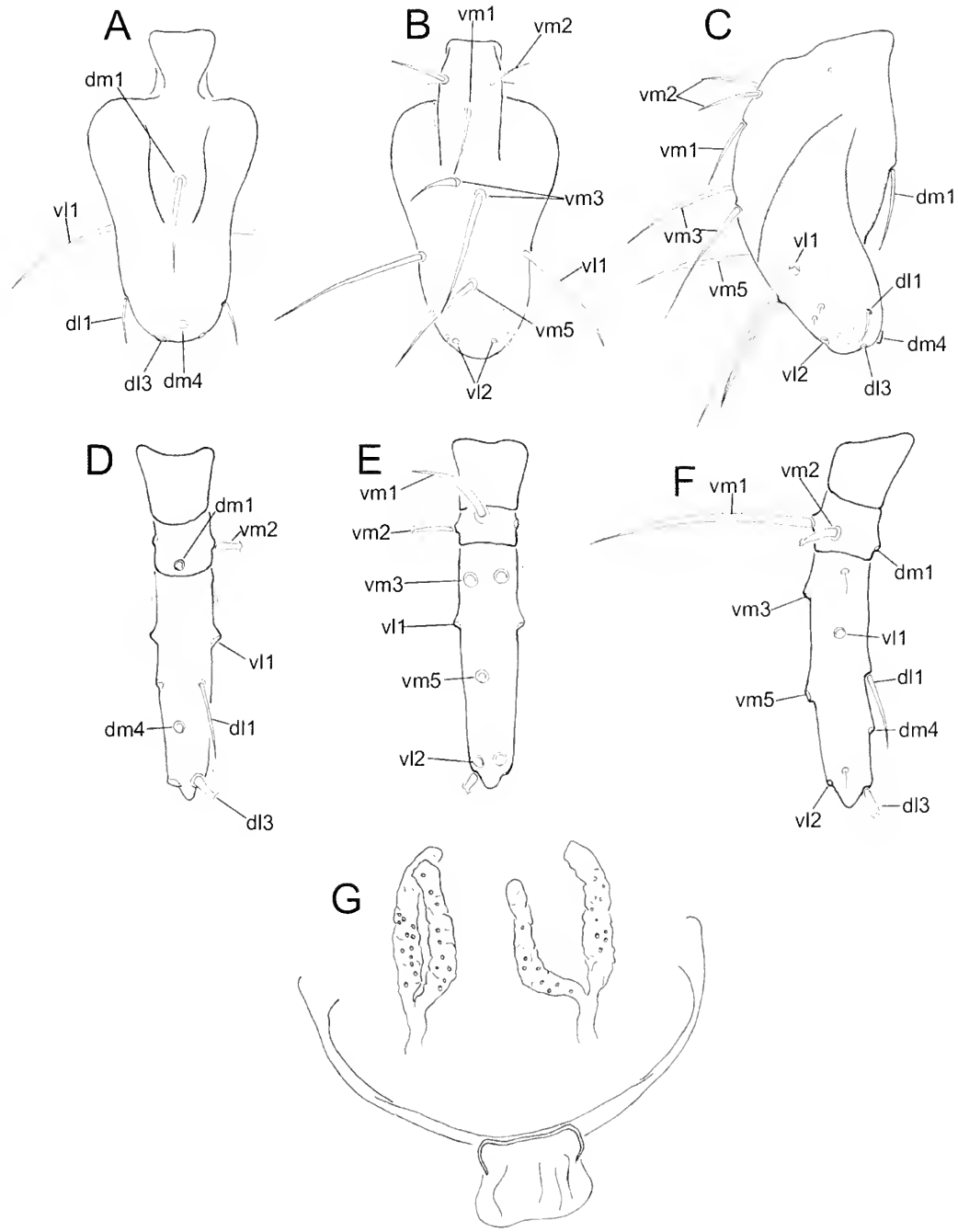

Figure 19.—*Paradraculoides trinity* sp. nov.: A–C, holotype male (WAM T92510): A. Flagellum, dorsal; B. Flagellum, ventral; C. Flagellum, lateral. D–G, paratype female (WAM T92515): D. Flagellum, dorsal; E. Flagellum, ventral; F. Flagellum, lateral; G. Spermathecae, ventral. Abbreviations: dm1, 4 (dorso-median 1, 4), dl1, 3 (dorso-lateral 1, 3), vm1, 2, 3, 5 (ventro-median 1, 2, 3, 5), vl1, 2 (ventro-lateral 1, 2).

close to posterior margin; dl1 between dm4 and vl1, but much closer to dm4; dl3 closer to posterior margin than vl2; vm2 situated anterior to vm1, vm1 closer to vm2 than to vm3; vm5 situated closer to vl2 than to vm3; 1 pair of microsetae near anterior end, six pairs posteriorly between dl1, dl3, vl1 and vl2. Female: 5.75 x longer than broad (Figs. 18H–J, 19D–F); seta dm1 situated towards posterior end of flagellomere II, more posterior than vm2; setae dl1 situated anterior to dm4, dm4 situated at three quarters length of flagellomere III; dl3 situated at posterior margin directly above vl2, vm1 situated slightly more anterior than vm2, vm3 situated closer to vm1 than to vm5, vm5 halfway between vm3 and vl2, vl1 situated posterior to vm3 and anterior to dl1; 1 pair of microsetae baso-laterally on flagellomere III, 1 pair of microsetae laterally between vl2 and dm4.

Female genitalia: Two pairs of elongate spermathecae, each pair connected basally before connection with bursa (Fig. 19G), distally round and smooth; sparsely covered with small pores; gonopod short, distally broad.

Dimensions (mm): Holotype male (WAM T92510): Body length 4.76. Propeltidium 1.38/0.75. Chelicera 0.99. Flagellum 0.58/0.33. Pedipalp: trochanter 0.65, femur 0.75, patella 0.79,

tibia 0.77, tarsus 0.37, claw 0.19, total excluding claw 3.33. Leg I: trochanter 0.46, femur 2.00, patella 2.54, tibia 2.02, metatarsus 0.60, tarsus 0.81, total 8.43. Leg IV: trochanter 0.50, femur 1.84/0.29, patella 0.77, tibia 1.34, metatarsus 0.96, tarsus 0.58, total 5.99.

Paratype female (WAM T92515): Body length 5.76. Propeltidium 1.54/0.86. Chelicera 1.12. Flagellum 0.46/0.08. Pedipalp: trochanter 0.69, femur 0.85, patella 0.87, tibia 0.79, tarsus 0.40, claw 0.17, total excluding claw 3.60. Leg I: trochanter 0.52, femur 1.82, patella 2.21, tibia 1.82, metatarsus 0.52, tarsus 0.75, total 7.64. Leg IV: trochanter 0.44, femur 1.73/0.29, patella 0.73, tibia 1.35, metatarsus 1.19, tarsus 0.67, total 6.11.

Variation: Body length (males, $n = 1$) 5.22, (females, $n = 1$) 4.35; propeltidium (males) 1.57/0.83, (females) 1.48/0.79.

Remarks.—*Paradraculoides trinity* is known from Trinity Bore, Western Australia (Fig. 1).

ACKNOWLEDGMENTS

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SHORT COMMUNICATION

Making the invisible visible: methods to enhance features of tiny spider webs

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Abstract. The characteristics and designs of webs provide valuable information on ecology, behavior and phylogenetic relationships. Characters are often obtained from detailed analyses of web photographs. We describe new methods to enhance web visibility; they consisted of painting the inner surfaces of Petri dishes with an opaque black spray paint that produced a rough surface, and then applying a salt spray from a nebulizer or fine white powder to tiny webs constructed in there. Using these methods on webs of *Oecobius concinnus* Simon, 1893, we discerned several unknown details of the refuge and cribellar threads that had previously gone undetected.

Keywords: Nebulizer method, tiny webs, *Oecobius concinnus*

Web designs and web building behavior have been useful to support and clarify phylogenetic relationships among higher taxonomic groups (e.g., families) and to delineate evolutionary patterns in web construction (Eberhard 1990; Agnarsson 2004; Lopardo et al. 2004; Lopardo & Ramírez 2007, Eberhard et al. 2008a; Kuntner & Agnarsson 2009; Eberhard & Barrantes 2015). Much of this information was derived from analyses of high-contrast web photographs that revealed web features and web construction behavior (Lopardo & Ramírez 2007; Barrantes et al. 2017).

Several techniques have been used previously to enhance the visibility of silk threads in spider webs (Comstock 1940; Langer & Eberhard 1969; Eberhard 1976; Carico 1977; Ramírez et al. 2013). Coating webs with cornstarch or talcum powder has been a useful technique to enhance the visibility of webs to allow taking photographs with enough contrast to measure fine details under both field (Eberhard 1976) and lab conditions (Barrantes & Eberhard 2012). However, detailed information on design and features of tiny webs (about 2 cm diameter), such as those of oecobiids, remains elusive, because particles of talcum powder or cornstarch are too large to allow resolution of many of the details.

The purposes of this paper are (1) to describe a new method of enhancing the visibility of fine features of the tiny webs, using *Oecobius concinnus* Simon, 1893 (Oecobiidae) as an example; and (2) to compare it with the talcum powder coating method (Eberhard et al. 2008b). *Oecobius* are known to construct tiny, oval to circular webs that include a tent (Hingston 1925), and an inner layer (carpet) that is closer to the ground and serves as a floor; the spider inhabits the space between the tent and the carpet. The tent is attached at several points to the ground, and spaces between them serve the spider as exits and entrances to the space covered by the tent. In addition, the web has long radial threads that extend outside of the carpet, and cribellate silk threads surrounding it (Glatz 1967), both nearly invisible to the naked eye.

We placed each of 15 adult females in Petri dishes that were previously painted with black paint (BBQ Black Ultra High Heat Spray, Harris Paints). This spray paint produced a fine-grained rough, irregular surface similar to sites where spiders build webs in the field. Each of five finished webs were placed inside a plastic box (length = 30 cm, width = 15 cm, height = 5 cm). We then applied a nebulizer (OMRON model NE-C801LA) twice for 3 min, with a pause of 10 min between applications, covering the box immediately after each application. The nebulizer contained a 0.2 M saline solution that produce a mist of 3 μm crystals in the box that gradually accumulated on the web, making its threads easier to see, measure, and photograph. The 10-min pause between applications was enough to allow the mist to deposit on silk threads and dry out, but we did test shorter intervals.

We applied talcum powder to another five webs (Eberhard et al. 2008b), then turned each Petri dish upside down and gently tapped its bottom repeatedly. Tapping the dish removed most of the talcum powder from the dish and from non-adhesive threads, but talcum adhered tightly to sticky threads. To coat webs with talcum powder, we put talcum inside a cloth bag made from a pair of socks (one inside the other), and then patted the bag gently from a distance of about 10 cm, as described by Eberhard (1976) for cornstarch. For the last five webs, we applied both methods in sequence; first the web was nebulized and then talcum coated. In this case, we also removed the talcum from the dish and non-adhesive threads as described above. We photographed each of the 15 webs with a Nikon Coolpix P100 and a Canon EOS Rebel T2i.

All webs were built at the junction between the floor and the wall of the Petri dish. The tent of *O. concinnus* had a length of 19.92 mm (SD = 2.54 mm), and a width of 8.25 mm (SD = 2.09 mm). Except for the tent, other parts of the web are nearly impossible to see in untreated webs, particularly the section of the tent connected to the substrate and threads laid around the margins of the tent.

The salt crystals made silk threads of the tent and carpet, as well as some radial and cribellate threads visible (Figs. 1 & 2). The threads attaching the tent to the substrate and the carpet, which was seen as a dense layer underneath the tent, could be discerned. Many threads of the tent and the carpet itself could also be distinguished using this technique (Fig. 1a, b). The coating of talcum powder made the cribellate threads around and on the carpet visible, but the talcum powder particles are too big to allow the individual resolution of these threads and had less resolution on the other web details that were visible with the saline solution (Fig. 1e). However, some details of threads, particularly of the carpet, were more difficult to discern in photographs taking after applying both methods in sequence (i.e., talcum powder after nebulizer), though radial threads appeared more evident (Fig. 1d).

For small webs like those of oecobiids, hahniids, erigonine linyphiids, and Mysmeninae (Hormiga et al. 2007), we suggest using each method (nebulizer and talcum powder) on different groups of webs. The application of saline solution will make all threads visible in fine detail, and the talcum powder will allow an easier discrimination of sticky threads. The threads of some small webs may also be further enhanced if both methods are applied in sequence (Fig. 1d). The nebulizer method has at least four advantages over the spray-painting method used by Richter (1970), when applied on tiny webs (GB unpubl. data). With the spray-painting method, threads are

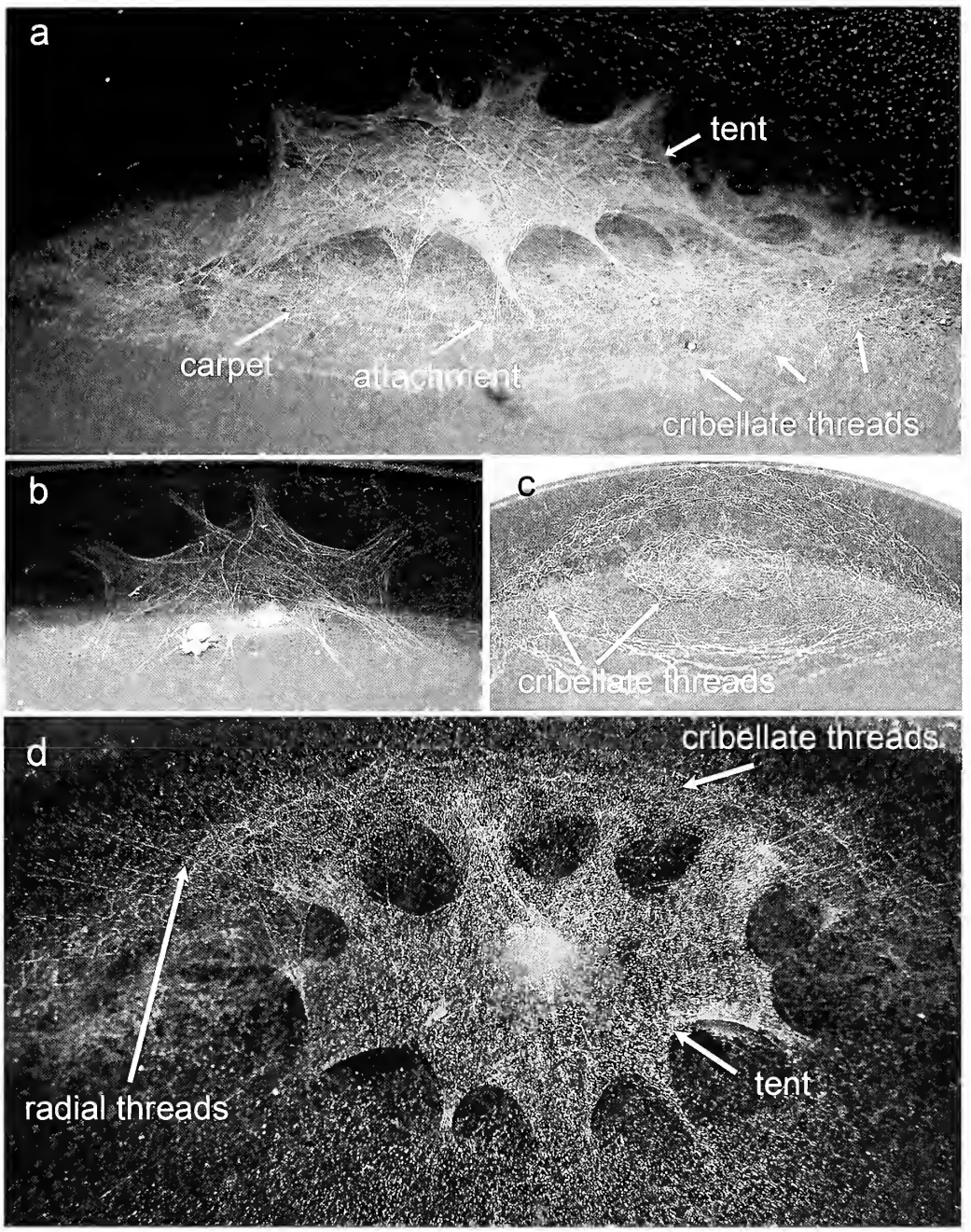

Figure 1.—Characteristics of the web of *Oecobius concinnus*. a- Different components of the web. b- Closeup of the web tent showing individual threads of the tent web. c- Band of cribellate threads around the tent and on the carpet. d- Features of web after applying the nebulizer and the talcum powder methods in sequence. We used the nebulizer method for a and b, talcum powder for c, and both methods for d.

more difficult to discern as they tend to stick to each other; the substrate is also painted, making contrast of threads more difficult; delicate webs could be damaged if spray is applied close to them; and spiders are often affected by paint. The nebulizer method is ecologically friendly and could potentially be applied in the field, but there are two possible limitations. Firstly, webs need to be enclosed right after each application, and this could be difficult in the field, particularly for larger webs. Secondly, the nebulizer has to be connected to an electric source, though this could be solved using a cordless nebulizer.

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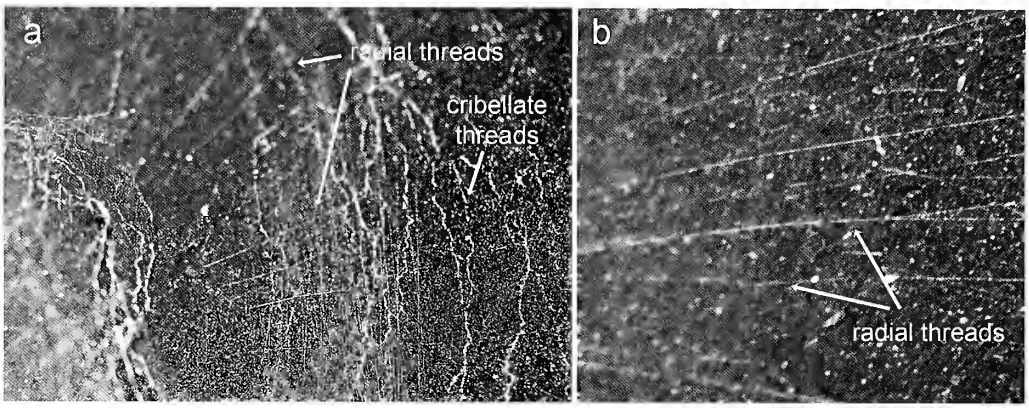

Figure 2.—Detail of radial threads seen with the salt crystal technique. a- Radial and cribellate threads near the tent. b- Detail of radial threads beyond the outer edge of the carpet.

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SHORT COMMUNICATION

Black widow spiders, *Latrodectus* spp. (Araneae: Theridiidae), and other spiders feeding on mammals

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Abstract. A survey of reports on spiders preying on small, non-flying mammals (i.e., mice, deer mice, voles, rats, heteromyid rodents, shrews) published in the literature and in the social media yielded a total of 42 naturally occurring incidents. Spiders from five families (Agelenidae, Ctenidae, Sparassidae, Theraphosidae, and Theridiidae) were reported capturing small mammals under natural conditions. Additionally, spiders from four more families (Atracidae, Lycosidae, Pisauridae, and Porrhothelidae) are known to kill small mammals in captivity. Approximately 80% of the reported incidents were attributable to theridiid spiders, especially the Australian redback spider (*Latrodectus hasselti* Thorell, 1870) and three species of North American widow spiders (*Latrodectus geometricus* C.L. Koch, 1841, *Latrodectus hesperus* Chamberlin & Ivie, 1935, and *Latrodectus mactans* (Fabricius, 1775)) that have been shown to be expert mouse-catchers. The success of widow spiders in subduing small mammals can be explained by their ability to spin strong webs made up of tough silk, and producing a very potent toxin (α-latrotoxin) specifically targeting the vertebrate nervous system.

Keywords: Comb-footed spiders, strong webs, vertebrate-specific toxin, predation, broad diets

While predation on frogs, lizards, snakes, fish, and birds by spiders has been extensively reported and discussed in the scientific literature (see McKeown 1943; McCormick & Polis 1982; Menin et al. 2005; Toledo 2005; Brooks 2012; Nyffeler & Pusey 2014; Walther 2016; Nyffeler et al. 2017a), predation on mammals has attracted much less attention – apart from the fact that many tarantula keepers feed their animals with small mice and that mice, rats, and guinea pigs are used as experimental animals to test the effects of spider venoms on the mammalian nervous system (Bücherl 1971; Marshall 2001). In the year 2016, an unusually large huntsman spider was filmed in Coppabella, Queensland (Australia), carrying around a presumably freshly killed mouse before attempting to eat it (see Whyte & Anderson 2017). Although skeptics may question the likelihood that a huntsman spider is capable of subduing and carrying around prey the size of a mouse, in our opinion this video is authentic, first because McKeown (1952) had already described an almost identical incident of a mouse-eating huntsman spider likewise witnessed in a house in Queensland, and second because large huntsman spiders (i.e., *Heteropoda* spp.) were observed killing and devouring vespertilionid bats and cane toads the size of small mice (see Nyffeler & Knörnschild 2013, and https://imgur.com/lwQxbSt). After the videographer had posted a video of this scene on facebook, the news media and social media spread the story around the world – and this inspired us to review the topic "spider predation on mammals" on a global scale. The senior author approached this task by conducting an extensive bibliographic search to identify all published reports of predation on small mammals by spiders using the ISI Web of Science Thomson-Reuters database, Scopus database, Google Scholar, Google Books, and Google Pictures. Social media sites were also searched for content indicating predation on small mammals by spiders.

In total, 42 reports of naturally occurring predation on small, non-flying mammals by spiders were found, about half of which had previously been published in the scientific or popular literature (Appendix 1). In addition to this, four reports dealing with staged events observed under laboratory conditions (spiders and small mammals being confined in cages) are included in Appendix 1. In about half of all documented naturally occurring incidents, evidence in the form of photos or videoclips was available. From the 42 documented incidents, 52% originate from the 21th century, 36% from the 20th century, and 12% from the 19th century. The rapid increase in the number of incidents reported since the beginning of the 21th century is most certainly because of the uploading of photos and media to the internet (compare Nyffeler et al. 2017b). In cases referring to unidentified web-building spiders, these could be classified as belonging to the family Theridiidae based on their prey capture behavior of (1) using extraordinarily strong, irregular webs capable of retaining prey of large size relative to the spider's size, (2) lifting prey organisms above the floor by the spider using spider silk, and (3) being small-sized spiders equipped with potent venom very effectively targeting the vertebrate nervous system (see Blackledge et al. 2005; Blackledge & Zevenbergen 2007; Garb & Hayashi 2013). In cases where popular literature was consulted, this referred to chance observations made between 1836 and 1926, that is, at a time when hardly anything on vertebrate-eating spiders was known that could have served as an inspiration to invent a story on mouse-catching spiders. Also, the fact that it had been mentioned already in these early reports that the spiders were lifting captured mice or rats some distance above the floor prior to killing them seems to us to be proof that these were authentic occurrences. In one of the popular reports, the predation event had been documented by a photo.

In this paper, we present incidents of spider predation (or predation attempts) on small mammals represented by mice (*Mus musculus*; Muridae), rats (e.g., *Rattus* sp.; Muridae), deer mice (*Peromyscus maniculatus* and *P. leucopus*; Cricetidae), voles (*Microtus californicus*; Cricetidae), shrews (*Microsorex hoyi*; Soricidae), and heteromyid rodents (Heteromyidae) (Appendix 1). In addition to this, a predation attempt by a spider on a mouse lemur (*Microcebus lehilahytsara*; Cheirogaleidae) was reported (see below). Furthermore, predation on immature hamsters (Cricetidae) by captive, large theraphosids is known (e.g., see online at https://www.youtube.com/watch?v=yanYZwO474E). In 86% of the naturally occurring incidents, the victims were deer mice, mice or rats (family Cricetidae and Muridae). In or near human dwellings in Australia, mammalian prey captured by the redback spider were found to be exclusively house mice, whereas the victims of North American widows were deer mice, rats, and probably also house mice. Even bats have been found entangled in North American widow webs (O'Meara 2011, p. 463); spider predation on bats, however, has been reported elsewhere (see Nyffeler & Knörnschild 2013). The exclusiveness of the capture of

Figure 1.—A theraphosid, *Aphonopelma chalcodes* Chamberlin, 1940, preying on a heteromyid rodent in Tucson, Arizona (photo by Michael Skinner).

house mice by spiders in or near houses in Australia might be explained by the fact that in this part of the world only non-native rodents (i.e., in particular house mice) closely associate with humans, while native Australian rodents very rarely get close to human settlings (B. Breed, pers. comm.; K. Rowe, pers. comm.; K. Vernes, pers. comm.).

Naturally occurring incidents of predation on non-flying mammals have been reported from the USA (22 incidents), Australia (16 incidents), and 1 incident each from India, the United Kingdom, Mexico, and Madagascar. Thus, reports from the USA and Australia account for ~90% of all cases (Appendix 1). In the USA, incidents of mammal predation by spiders have been reported from the Northeast (Maryland, Massachusetts), the South (Alabama, Florida, Kentucky, Louisiana), the Midwest (Indiana, Ohio), and the West (Arizona, California, Colorado, Oregon), thus, one can say from throughout the country (see references in Appendix 1).

Spiders from five families (Agelenidae, Ctenidae, Sparassidae, Theraphosidae, and Theridiidae) were reported capturing small mammals under natural conditions. In addition to this, spiders from four families (Atracidae, Lycosidae, Pisauridae, and Porrhothelidae) have been documented killing small mammals in captivity (see online at https://www.youtube.com/watch?v=fGZ9jOrVwk0; Schmidt 1953, 1957; Kaston 1965; Bücherl 1971; Laing 1975). Furthermore, a mouse lemur (*Microcebus lehilahytsara* Roos & Kappeler, 2006) was trapped in a large, dense web constructed by a spider of an unspecified taxon; this lemur, however, was rescued by human observers before the spider had the opportunity to kill and devour it (Crane & Goodman 2013). Because the lemur was inextricably entangled in the strong web, it ultimately would have died of starvation and desiccation regardless of whether the spider killed it by envenomation or not. Based on a photo of the web, the unidentified web owner was suspected to be either a theridiid or a large pisaurid (Crane & Goodman 2013), and since spiders from these families are known to prey on small mammals (see McKeown 1943, 1952; Schmidt 1953, 1957), it is likely that the spider in question would have killed and devoured the lemur, had this one not been rescued. In Western Australia, a marsupial mouse (*Antechinomys* sp.; Dasyuridae) was caught in a trapdoor spider's burrow (probably Idiopidae) but nothing is known about whether the spider was attacking the captive (Fraucca 1982). Idiopids are known to occasionally kill and consume vertebrate prey (Butler & Main 1959; Main 1996). Only 12% of the naturally occurring predation events were attributable to web-less hunting spiders. These refer to two incidents in which huntsman spiders (Sparassidae) were feeding on mice in houses in Queensland, Australia, one incident from India where a *Poecilotheria regalis* Pocock, 1899 (Theraphosidae) was found devouring a rat, one incident from Arizona (USA) where an *Aphonopelma chalcodes* Chamberlin, 1940 (Theraphosidae) was seen feeding on a heteromyid rodent (see Fig. 1), and one report from South America according to which *Phoneutria* sp. (Ctenidae) is occasionally feeding on rats (Bücherl 1971). These web-less spiders are powerful, ferocious predators weighing ≥10 g in the case of the theraphosids and >2 g in the case of the huntsman and ctenid spiders (Carrel 1987; Rind et al. 2011; Lapinski & Tschapka 2013). The question arises as to why records of mammal predation by web-less spiders are so scarce. This might be explained at least in part by difficulties in witnessing the feeding activities of web-less spiders in the wild, as compared to the more easily observed synanthropic web-building spiders (e.g., *Latrodectus* spp.). Ctenid, sparassid, and theraphosid spiders are predominantly nocturnal and therefore difficult to observe while hunting prey during the hours of darkness. In addition, theraphosids often feed in their burrows, out of human sight (Nyffeler et al. 2017e). Apart from mammals, additional vertebrates such as frogs, toads, lizards, snakes and—in the case of the theraphosids—even birds are preyed upon by web-less spiders (Bücherl 1971; Menin et al. 2005; Vieira et al. 2012; Nyffeler & Knörnschild 2013; Borges et al. 2016; Neogi & Islam 2017). At least in the case of the theraphosids and ctenids, it is proven that these spiders are equipped with potent venoms targeting invertebrate and vertebrate nervous systems (Bücherl 1971; Isbister et al. 2003; Garcia-Arredondo et al. 2015). In cage experiments, it has been shown that within 24 hours, a hungry theraphosid can reduce a mouse to nothing but a hard, dry mass of skin, hair, and bones (Rau 1931).

Figure 2.—Dead rat (*Rattus* sp.) trapped in the web of a brown widow, *Latrodectus geometricus* C. L. Koch, 1841, on a building in North Port, Florida (photo by Linda Nau).

The remaining predation events were attributable to web-building spiders, with the comb-footed spider family (Theridiidae) accounting for the vast majority of the documented incidents (roughly 80%; Fig. 2). Among these, the following species have been identified: the Australian redback spider (*Latrodectus hasselti* Thorell, 1870), three species of North American widow spiders (*Latrodectus geometricus* C. L. Koch, 1841, *Latrodectus hesperus* Chamberlin & Ivie, 1935, and *Latrodectus mactans* (Fabricius, 1775)) and the cosmopolitan house spider *Parasteatoda tepidariorum* (C. L. Koch, 1841). All these spiders are synanthropic, occurring in or near human dwellings, that is, in places such as houses, garages, garden sheds, barns, on outside furniture, farm equipment etc. (Garb et al. 2004). Indoors, they are often found in the corners of cupboards, behind furniture or under desks. Accordingly, all documented incidents of the capture of small mammals by this type of spiders occurred in or near human dwellings in urban, suburban or rural areas. Black widows accounted for roughly 60% of the reported incidents of predation on small mammals (Appendix 1).

The four widow species reported in our study are of similar size (usually weighing ~0.35-0.50 g as adult females; McKeown 1943; Anderson 1994; Shao & Vollrath 1999) with maximum weights of up to ~0.90 g (Anderson 1994). Exclusively females of *Latrodectus* spp. are engaged in the killing of small mammals, which can be explained by the fact that only full grown female spiders have large enough venom glands to produce a sufficient amount of venom needed to successfully envenomate a mouse or rat (i.e., sexual dimorphism in body size; Rash & Hodgson 2002). Black widow spiders construct irregular, three-dimensional space webs composed of extraordinarily tough silk, from which vertical sticky gum-footed threads extend to the floor (Blackledge et al. 2005; Blackledge & Zevenbergen 2007). These webs, located ~10-100 cm above the floor, are very strong, enabling the spiders to capture prey many times larger and heavier than themselves (see Shao & Vollrath 1999; Blackledge et al. 2005; Swanson et al. 2006). When a small mammal walks into such a web, it gets stuck to the sticky threads. Alerted by the prey-generated web vibrations, the spider rushes to the victim attempting to immobilize it by throwing with its hind legs sticky silk masses over it (Vollrath 2000). Once this has been accomplished, the spider administers one or several venomous bites thereby injecting a very potent vertebrate-specific toxin (α-latrotoxin) that is highly lethal to small mammals (Gendreau et al. 2017). The spider bites its victim either at the base of the tail where the skin is tender or on another soft spot such as the nose (e.g., Baerg 1954; YouTube videos cited in Appendix 1). Subsequently, the spider pulls its victim off the ground, raising it between 8 and 20 cm above the substrate (see Clagget 1914; McKeown 1943, 1952). In one study, a mouse was dead about 3 hours after its entrapment in a black widow spider web (Clagget 1914). For comparison, mice bitten by adult *L. tredecimguttatus* (Rossi, 1790) and *L. mactans* spiders in laboratory experiments were killed within ~20 minutes (Zumpt 1968; Maretić 1987). Black widows have been observed to not only kill mice but to also actually feed on them (see McKeown 1943). In several instances, the full predation process (mammal becoming entangled, swathed in silk, bitten, and suspended in the web by the spider) was witnessed by the reporting authors (e.g., Blair 1934), and in most YouTube videos dealing with this topic (see Appendix 1), the mice snared in spider webs were still alive at the time of filming, indicating that the incidents reported in this paper were in most cases real predation events and not cases of scavenging. In the United Kingdom, Felton (1968) reported a case in which a house mouse got stuck after falling down through a series of *Tegenaria* cob webs placed on top of one another. In this latter case, there is no evidence that the mouse was attacked and consumed by the spider so that this presumably was a case of accidental death by web entanglement. The victims were usually immature mice or rats of small size and in one instance an adult pygmy shrew of small size (Clagget 1914; Saunders 1929; Blair 1934; D'Amour et al. 1936; McKeown 1943). One immature mouse trapped and killed in a *L. hasselti* web weighed 4.7 g which was 14.4 times the spider's body mass (McKeown 1943). For comparison, fishing spiders of the genera *Dolomedes* Latreille, 1804 and *Nilus* O. Pickard-Cambridge, 1876 (Pisauridae), with a body mass of 0.5–2 g, can catch fish prey up to 4.5 times the spider's body mass (Nyffeler & Pusey 2014).

The potency of *Latrodectus* venom on mammals would indicate that it is more than capable for the spiders to have the potential to subdue mammals with their toxic bites. Venom from the Eurasian *L. tredecimguttatus* has an LD_{50} of 0.013 mg of dried gland extract per mouse translating to an overall LD_{50} of 0.9 mg/kg (Bettini & Marioli 1978). It is estimated that the venom of one spider had enough potency to kill 40 mice (Maretić & Lebez 1979). Venom from four species of Argentinian *Latrodectus* spiders produced LD_{50} values ranging from 3.1 to 22.5 µg/animal in 18–22 g CF-1 mice (de Roodt et al. 2017) translating to approximately 0.15 to 1.23 mg/kg for the average 20 g mouse. Using whole gland extract, D'Amour et al. (1936) estimated the LD_{50} in rats as 0.032 mg which they considered as 25% of the widow's venom quantity. As they used rats of 50 to 60 g weight, this would translate to an LD_{50} of 0.53 to 0.64 mg/kg. These LD_{50}s are similar to that for American rattlesnakes (Glenn & Straight 1978). Autopsy of mammals (e.g., rats, cats, mice) injected with *Latrodectus* venom in the lab exhibit multiple organ aberrations with edema (swelling) and hyperemia (increased blood flow to tissues) being common (Maretić & Lebez 1979).

The house spider *Parasteatoda tepidariorum* constructs the same web type as the black widow spiders and the prey capture behavior of

these two spider groups is essentially the same (see Ewing 1918). Like the black widows, *P. tepidariorum* pulls prey off the ground, raising them ~8–10 cm above the substrate (e.g., McCook 1889; Davis et al. 2017). Nonetheless, as Appendix 1 reveals, *P. tepidariorum* apparently is much less successful in catching small mammals. This may be due to the fact that the species is considerably smaller and weaker than *Latrodectus* spp., with a body mass of ~0.05–0.17 g (Anderson 1994; Boutry & Blackledge 2008) and lacks the vertebrate-specific toxin (α-latrotoxin; Gendreau et al. 2017). The lower potency of the *P. tepidariorum* toxin seems to be evidenced by the fact that it took a mouse at least ten hours to die after being trapped and bitten by a *P. tepidariorum* (see McCook 1889).

Apart from preying on small mammals, black widows have been reported to also capture and devour other types of vertebrates including amphibians, reptilians, and birds (e.g., Raven 1990; Anderson 2011; Brooks 2012; Metcalfe & Ridgeway 2013; Shine & Tamayo 2016; Rocha et al. 2017). So far, 9 different *Latrodectus* spp. (*L. geometricus*, *L. hasselti*, *L. hesperus*, *L. katipo* Powell, 1871, *L. lilianae* Melic, 2000, *L. mactans*, *L. pallidus* O. P.-Cambridge, 1872, *L. revivensis* Shulov, 1948, and *L. tredecimguttatus*) in various geographic regions such as Australia, Brazil, Canary Islands, Croatia, Dominican Republic, Israel, Italy, Mexico, New Zealand, Romania, South Africa, Spain, and USA have been reported to be engaged in preying on vertebrates (e.g., Blair 1934; Newlands 1978; Schwammer & Baurecht 1988; Blondheim & Werner 1989; Hódar & Sánchez-Piñero 2002; Lettink & Patrick 2006; Jones et al. 2011; Colombo 2013; Hamilton et al. 2016; Shine & Tamayo 2016; Rocha et al. 2017). The fact that preying on vertebrates by *Latrodectus* spp. apparently is widespread and not uncommon, is strong evidence for the ecological significance of α-latrotoxin as a vertebrate-specific toxin. It is unlikely that α-latrotoxin evolved as a defensive compound due to the difficulty of inflicting a bite from small fangs to the minuscule amount of exposed dermal area of an attacking mammalian predator protected by a coat of fur. When the western black widow, *L. hesperus*, was attacked by *Peromyscus* mice in laboratory trials, the spiders responded by expelling sticky aggregate gland silk, which was an efficacious, physically irritating repellent that increased spider survival (Vetter 1980). Additional evidence arguing for the purposeful evolution of a mammalian-specific *Latrodectus* venom component is the specificity of these components. Currently, seven latrotoxins have been isolated from *L. tredecimguttatus*; two are latroinsectotoxins which are strongly deleterious to insects but innocuous for vertebrates, a latrocrustatoxin which affects crustaceans but not insects or mammals and α-latrotoxin which causes trauma in many mammals but has no effect on insects or crustaceans (Ushkaryov et al. 2004). Black widows *Latrodectus* spp. and the house spider *P. achaearanea* are generalist predators which predominantly feed on arthropods such as ants, beetles, and even scorpions (D'Amour et al. 1936; Nyffeler et al. 1988; Hódar & Sánchez-Piñero 2002). However, it seems quite remarkable that, considering how rare it probably is for a widow spider to subdue a mammal or other vertebrate, that there would be sufficient evolutionary pressure to generate a venom component specifically for this purpose. Their capability to additionally subdue mammals and other vertebrates broadens their diets, and this is presumed to improve the survival of these spiders (also see Nyffeler et al. 2017a,c).

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Appendix 1.—Spiders predaceous on small mammals (42 records from the field and 4 records from spiders in captivity). * Unidentified web-building spiders have been classified as theridiids based on their reported prey capture behavior. ** Indicates predation attempts (a victim inextricably entangled in a spider web was freed by human observers). Type of evidence: OE = Observational evidence; P = Photo; V = Video. Type of prey: DM = Deer mouse; H = Hamster; HM = House mouse; HR = Heteromyid rodent; ML = Mouse lemur; M = cited as "mouse"; R = Rat; S = Shrew; VO = Vole; A = ambiguous (DM or HM).

Predator taxon	Country	Type of evidence	Type of prey	Source
MYGALOMORPHAE				
Theraphosidae				
Aphonopelma chalcodes Chamberlin, 1940	USA	P	HR	Social Media[A]
Grammostola rosea (Walckenaer, 1837)	In captivity	P	S	Social Media[B]
	In captivity	V	H	Social Media[C]
Poecilotheria regalis Pocock, 1899	India	OE	R	Pocock 1899
ARANEOMORPHAE				
Agelenidae				
Tegenaria domestica (Clerck, 1757)	UK	OE	HM	Felton 1968
Ctenidae				
Phoneutria **sp.**	Brazil	OE	R	Bücherl 1971
Lycosidae				
Unspecified	In captivity	OE	M	Kaston 1965
Pisauridae				
Dolomedes fimbriatus (Clerck, 1757)	In captivity	OE	HM	Schmidt 1953, 1957
Sparassidae				
Unspecified	Australia	OE	M	McKeown 1952
	Australia	P, V	M	Whyte & Anderson 2017
Theridiidae				
Latrodectus geometricus C. L. Koch, 1841	USA	V	DM	Social Media[D]
	USA	P	R	Social Media[E]
Latrodectus hasselti Thorell, 1870	Australia	V	HM	Social Media[F]
	Australia	V	HM	Social Media[G]
	Australia	V	HM	Social Media[H]
	Australia	P	HM	McKeown 1943
	Australia	P	HM	McKeown 1952
	Australia	OE	HM	McKeown 1952
	Australia	P	HM	Social Media[I]
	Australia	P	HM	Social Media[J]
	Australia	V	HM	Social Media[K]
	Australia	V	HM	Social Media[L]
	Australia	V	HM	Social Media[M]
	Australia	OE	HM	Social Media[N]
	Australia	P	HM	Social Media[O]
Latrodectus hesperus Chamberlin & Ivie, 1935	USA	OE	M	Anderson 2011
	USA	OE	M	D'Amour et al. 1936
	USA	P	M	Heyn 1940
	USA	OE	M	Social Media[P]
	USA	OE	VO	Neitzel 1965
Latrodectus mactans (Fabricius, 1775)	USA	OE	M	Blair 1934
Latrodectus sp.	USA	V	DM	Social Media[Q]
	Mexico	V	DM?	Social Media[R]
Latrodectus sp.?	USA	OE	M	Clagget 1914
	USA	OE	M	Clagget 1914
Parasteatoda tepidariorum (C. L. Koch, 1841)	USA	OE	M	McCook 1889
Parasteatoda tepidariorum?	USA	P	M	Popular Magazine[S]
Unidentified theridiids*	USA	OE	M	Popular Magazine[T]
	USA	OE	M	Popular Magazine[U]
	USA	OE	M	Popular Magazine[V]
	USA	OE	R	Popular Magazine[W]
	USA	P	A	Social Media[X]
	USA	V	DM?	Social Media[Y]
	USA	V	A	Social Media[Z]

Appendix 1.—Continued.

Predator taxon	Country	Type of evidence	Type of prey	Source
Unidentified				
Unidentified web-builder	USA	OE	S**	Saunders 1929
Unidentified (Theridiidae or Pisauridae?)	Madagascar	P	ML**	Crane & Goodman 2013

[A] https://www.flickr.com/photos/12921146@N04/3862145569
[B] https://www.scienceesource.com/CS.aspx?VP3=SearchResult&ITEMID=SS2286340
[C] https://www.youtube.com/watch?v=yanYZwO474E
[D] https://www.youtube.com/watch?v=IMWQxlwjiFo
[E] https://www.flickr.com/photos/15250800@N03/7986061905/in/photostream/
[F] https://www.youtube.com/watch?v=vMh50PRCLvI
[G] https://www.youtube.com/watch?v=rl2CngnT_sw
[H] https://www.youtube.com/watch?v=R-uC2gr97uU
[I] http://www.over50sforum.com/showthread.php?p=868784
[J] http://www.abc.net.au/news/2016-02-11/spider-vs-mouse/7158520
[K] https://www.youtube.com/watch?v=dfriTzNhuvg
[L] https://www.youtube.com/watch?v=kaUQj3NE0FQ
[M] Video posted on the 'YouTube' website https://www.youtube.com/watch?v=V4rzICIWQUE but subsequently removed.
[N] https://answers.yahoo.com/question/index?qid=20100804140057AAPZUc1
[O] http://gardenglut.blogspot.ch/2012/02/weird-scenes-inside-backshed.html
[P] http://www.mypmp.net/2015/07/01/pest-trends-brown-widow-knows-how-to-deal-with-unusual-prey/
[Q] https://www.youtube.com/watch?v=rikAI_V2y1M
[R] https://www.youtube.com/watch?v=9SVwP2vo86o
[S] Technical World Magazine Vol. 12, No. 1, p. 696 (September 1909)
[T] Nature Magazine Vol. 7-8, p. 58 (1926)
[U] Fancier's Journal & Poultry Exchange Vol. 3, p. 363 (August 7, 1876)
[V] Popular Science Monthly Vol. 40, pp. 575-576 (February 1892)
[W] New England Farmer Vol. 14, p. 32 (1836)
[X] http://bythedrop.com/gallery/insects/spiders/Mouse-Caught-in-Spider-Web-Ohio
[Y] https://www.youtube.com/watch?v=cBkhQh5gOqo
[Z] https://www.youtube.com/watch?v=kgT35ej1YDA

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SHORT COMMUNICATION

Comparative reproductive output of two cellar spiders (Pholcidae) that coexist in southwest Ohio

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Abstract. Species differ in their reproductive abilities, which may affect their success. In southwest Ohio, populations of *Pholcus phalangioides* (Fuesslin, 1775) (Araneae: Pholcidae) have largely been replaced by *Pholcus manneli* Gertsch, 1937 (Araneae: Pholcidae). We suspected that differences in reproductive output underpinned the success of *P. manneli*. We documented the reproductive success of both species in the laboratory. Female *P. phalangioides* mated more readily and were more likely to produce eggsacs than *P. manneli*, but the timing of eggsac production and spiderling emergence were similar. The eggsacs of *P. phalangioides* contained smaller numbers of larger eggs but similar numbers of spiderlings emerged from the sacs of both species. We uncovered a negative relationship between egg size and number for *P. phalangioides*, but not for *P. manneli*. Our results do not explain the relative success of *P. manneli*, however, we have uncovered differences in the reproductive strategies utilized by these ecologically-similar congeners.

Keywords: Invasive species, tradeoffs, reproduction

Limited resources dictate that animal reproductive strategies involve tradeoffs where investment in one beneficial fitness parameter precludes the full development of other aspects (Saeki et al. 2014). One example is the spider *Loxosceles rufescens* (Dufour, 1820), whose slow heart rate, low metabolism, and long lifespan facilitates its dispersal around the world. However, these same features mean it has limited mobility and often does not spread from the initial point of introduction (Nentwig et al. 2017). Common tradeoffs that are documented include those between size and number of eggs or offspring (Macip-Ríos et al. 2012), number of offspring and the growth or survival of those offspring (Sikes 1998; Cronin et al. 2016), and investment in current vs. future reproductive success (Rios-Cardenas et al. 2013). The decision to allocate energy or effort in one way or another can determine the success of populations and specific differences in reproductive strategies or reproductive potentials may be what allows one species to displace another or to buffer a species against displacement or extinction (Allen et al. 2017).

Historically, *Pholcus phalangioides* (Fuesslin, 1775) was the dominant pholcid species in southwest Ohio but, over the last 10 years, *Pholcus manneli* Gertsch, 1937 has displaced *P. phalangioides* and established robust populations across the region (A.L. Rypstra, personal observation). As congeners, the two species are superficially similar in appearance and both species build their irregular tangle webs under ledges and in the corners of buildings and barns (Jackson & Brassington 1987; Cutler 2007). However, adult, *P. phalangioides* are much larger (>6 mm in body length) than adult *P. manneli* (<5 mm in body length) (Cutler 2007). In general, the global success of *P. phalangioides* has been attributed to its behavioral flexibility, ability to invade the webs of other species (Jackson & Brassington 1987; Jackson 1992), the subdivision and genetic structure of its populations (Schäfer et al. 2001), and the plasticity in growth and body size (Miyashita 1988a, b; Uhl et al. 2005; Wilder 2013). Essentially nothing is known about the biology of *P. manneli*, especially the traits that are enabling it to take over areas with well-established *P. phalangioides* populations.

The purpose of this study was to document aspects of the reproductive output of *P. phalangioides* and *P. manneli* to determine if they could account for the success of *P. manneli* in displacing *P. phalangioides*. If *P. manneli* had higher reproductive output than *P. phalangioides* found in the same area, that difference could explain why *P. manneli* was able to replace *P. phalangioides*. Specifically, we predicted that the *P. manneli* would produce more offspring in a shorter time frame than *P. phalangioides*. In addition, we expected that the *P. manneli* would not need to make a trade-off between egg size and number that would be evident for *P. phalangioides*.

Spiders of both species were collected from buildings and barns around Butler and Hamilton Counties in southwest Ohio, USA between September 2013 and September of 2017. We housed spiders individually in cylindrical translucent plastic containers; those for *P. manneli* were 9 cm tall with a diameter of 13 cm and those for *P. phalangioides* were 12 cm tall with a 15 cm diameter. We kept the spiders in a climate-controlled room set on a 12:12 L:D cycle, 25°C, and 50% humidity and fed them two crickets, *Gryllodes sigillatus*, approximately equal in length to the spider (3 mm or 6 mm) once per week. We mated spiders by adding a male to a container where a female was housed and monitored the interaction. If the spiders acted aggressively toward one another, the male was removed. If mating occurred, we waited until the male and female separated and removed the male. The females were monitored so that the appearance of an eggsac and the emergence of spiderlings could be recorded.

As part of our effort to maintain laboratory populations of these species, we regularly attempted to mate animals in the laboratory. Of these, 41 pairs of *P. manneli* and 44 pairs of *P. phalangioides* were known to have molted to adulthood in the laboratory and, thus, were virgins. In all cases, the animals were mated 2–3 weeks after completing the final molt. After mating, we monitored the females for 30 days and recorded eggsac production and spiderling emergence. The number of females of each species that produced eggsacs as well as the number of those eggsacs that successfully produced spiderlings were compared using Fisher exact tests. The number of spiderlings emerging from eggsacs were compared using the Mann Whitney U Test.

Only 16 of the 41 *P. manneli* virgins we mated produced eggsacs, which was significantly less than the 40 of the 44 *P. phalangioides* virgins who deposited eggs (Table 1). Some spiderlings emerged from all of the *P. manneli* eggsacs but spiderlings appeared from only 34 of the 40 eggsacs produced by *P. phalangioides* (85%) (Table 1). The number of spiderlings per sac was highly variable for both species, ranging from 1 to 60 for *P. phalangioides* and 1 to 55 for *P. manneli*, and there was no overall difference between the two species in offspring number (Table 1).

The low reproductive output of virgin *P. manneli* in these initial observations was not supportive of our original hypothesis. Nevertheless, we noted that adults of both species are long lived (2–3 years

Table 1.—The reproductive output of *Pholcus manueli* and *Pholcus phalangioides* in our study. Values include the actual count or the average ± the standard error.

Category	*Pholcus manueli*	*Pholcus phalangioides*	Test Statistic	DF	P-value
Mating of virgin spiders					
Total number mated	41	44			
Number producing eggsac (%)	16 (39%)	40 (91%)	Fisher		<0.0001
Number of spiderlings emerged	16.1±4.2	18.6±3.5	Z=0.57	1	0.5650
Number eggsacs to hatch (%)	16 (100%)	34 (85%)	Fisher		0.1676
Mating of field caught adults					
Total number mated	66	38			
Number producing eggsac (%)	38 (58%)	29 (76%)	Fisher		0.0597
Time to 1st eggsac (d)	17.0±2.2	13.7±1.52	Z=0.07	1	0.9418
Number of 1st eggsacs hatched (%)	22 (58%)	21 (72%)	Fisher		0.3047
Time to hatch (d)	13.1±0.6	15.0±1.1	Z=1.42	1	0.1558
Number of spiderlings emerged from 1st eggsac	21.5±2.9	19.2±3.2	Z=0.66	1	0.5065
Number producing additional eggsacs (%)	13 (34%)	18(62%)	Fisher		0.0285
Time between 1st and 2nd eggsacs	23.8±6.4	22.7±8.7	Z=0.86	1	0.4100
Number of 2nd eggsacs to hatch (%)	3 (23%)	5 (28%)	Fisher		1.000
Egg data of field caught adults					
Sample size	17	19			
Clutch size	36.4±1.8	24.4±1.9	Z=3.57	1	0.0004
Egg diameter	0.57±0.01	0.83±0.01	Z=5.17	1	<0.0001

as adults in the laboratory) and reproductive adults can be found in our field sites during all months of the year (A.L. Rypstra and A. D. Berry, personal observation). In addition, individuals of both species willingly mate repeatedly in the laboratory (A.L. Rypstra and A. D. Berry, pers. observ.). While nothing is known regarding the mating system of *P. manueli*, sperm mixing and last male sperm priority seem to be the rule for other pholcid species (Uhl 2000; Schäfer & Uhl 2002; Calbacho-Rosa et al. 2013). Notably, the mating system of *P. phalangioides* involves multiple sequential matings (Uhl 2000; Schäfer & Uhl 2002) and females have a preference for experienced males (Hoefler et al. 2010). Given this background on pholcid mating, we considered the reproductive success of mature animals who likely had mated in the field before they were brought to the laboratory. We reasoned that their nutritional status and condition would be more reflective of what led to their relative success in the field and we hoped that documenting the reproductive success of these middle-aged animals would give us another angle on the differences between these two species.

Adult spiders were collected from area buildings and barns between September 2016 and March 2017 and housed in the laboratory for at least one week. We mated 66 *P. manueli* and 38 *P. phalangioides* pairs by introducing the male into the container housing the female, identical to the ones described above. If we observed aggression, we removed the male. If the spiders did not begin to mate within 20 minutes, we removed the male and introduced a new one from our laboratory population. If the second pair did not mate, then the female was returned to the population stored in the lab and we offered her the opportunity to mate again several days later. In this way, we eventually succeeded in getting all spiders to mate. The spiders were left alone until mating had completed, after which the male was removed and returned to his home container. We then returned both spiders to the environmental chamber described above. We checked females for eggsacs every day and recorded if and when the female produced an eggsac, the date spiderlings emerged, and the number of spiderlings that emerged. Once the eggsacs had hatched, we left the spiderlings with the mother until they had molted once, typically 7–10 d after emergence, and removed them. We continued to monitor the females until death and recorded the same information on all subsequent eggsacs. We compared the likelihood that one or more eggsacs (2–4) were produced, and the frequency with which spiderlings emerged from eggsacs of each species using Fisher exact tests. We compared the time it took for the spiderlings to emerge, the time between eggsacs (the time from the emergence of spiderlings from one eggsac to the production of the next), and the number of live spiderlings produced by each eggsac with Mann-Whitney U tests.

There were only a few differences in the reproductive parameters we documented (Table 1). *Pholcus phalangioides* females were more receptive as it took only 47 mating attempts to get the 38 field caught females mated (81%) whereas it took 149 trials to secure 66 mated *P. manueli* females (44%). *Pholcus phalangioides* appeared to be more likely to produce an eggsac than *P. manueli* females but the difference was not significant at the $P = 0.05$ level (Table 1). However, the established species was significantly more likely to produce a subsequent eggsac than the new arrival (Table 1). Neither the timing of eggsac production nor the time between its appearance and the emergence of spiderlings were different between the two species (Table 1). Similarly, the likelihood that the eggsac hatched and the number of spiderlings emerging from the eggsacs did not differ significantly between *P. manueli* and *P. phalangioides* (Table 1). Between 5 and 29 spiderlings emerged from *P. phalangioides* sacs and between 6 and 43 from *P. manueli* and there were no species differences in the production of live offspring (Table 1).

In order to determine if there was a trade-off between the number of eggs produced and the size of the eggs, we collected another 17 *P. manueli* and 19 *P. phalangioides* between October 2015 and April 2017. Three of these females (1 *P. manueli* and 2 *P. phalangioides*) produced eggsacs before we had the opportunity to mate them. We mated the rest of the females using the procedures described above. Five days after the eggsac appeared, we removed it and opened the sac to separate the eggs. We counted the eggs in each eggsac and randomly selected 7 eggs from each sac. We measured the egg diameter using a filar micrometer with an accuracy of 0.01 mm attached to a dissecting microscope (Wild Heerbrugg, Switzerland). We calculated an average egg size for each clutch. We compared egg size and egg number between species in separate Mann-Whitney U tests. We explored the effects of egg size and the species involved on the number of eggs in an eggsac using Poisson regression. We conducted the regression with and without the three eggsacs that were produced from field matings.

Pholcus manueli produced larger clutches than *P. phalangioides* but *P. phalangioides* clutches contained larger eggs (Table 1). Interestingly, clutch size was related to egg size in our Poisson regression (R^2

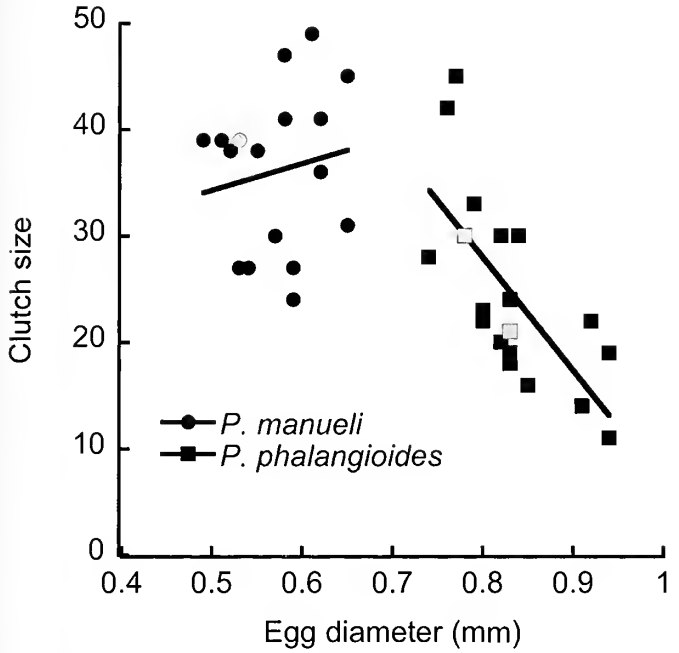

Figure 1.—The relationship between egg number and egg size for field caught adult *Pholcus phalangioides* and *Pholcus manueli*. Most data were collected from the first clutch produced after animals were mated in the laboratory. Gray symbols are data points for eggsacs produced by field-caught animals before they had the opportunity to mate in the laboratory.

= 11.47, $P = 0.0007$), however the species producing the eggsac was not significantly related to egg number in this analysis ($\aleph^2 = 0.10$, $P = 0.7484$). Instead, there was a highly significant interaction between the species of the female producing the eggsac and the size of the eggs it contained ($\aleph^2 = 18.6$, $P < 0.0001$). This interaction emerged because there was a negative relationship between egg size and egg number for *P. phalangioides* but there was no correlation between egg size and egg number for *P. manueli* (Fig. 1). This relationship was strong and was not affected by the data from the three individuals whose eggsacs resulted from mating that occurred before we collected them (Fig. 1).

These results hint at some differences in the reproductive strategies for these two congeners yet the differences do not necessarily help us understand how *P. manueli* has managed to replace *P. phalangioides* throughout our region. Female *P. phalangioides* readily mated in the laboratory and, overall, they were much more likely to produce eggsacs after a single mating than were *P. manueli* females. In addition, *P. phalangioides* females were more likely to produce multiple eggsacs and, even though the success of these later eggsacs was low, the offspring produced would still contribute to their population size. Thus, the laboratory data we collected from virgin and field collected adult individuals makes it difficult to argue that reproductive success can account for the success that *P. manueli* has had in displacing *P. phalangioides*.

Although *P. manueli* females produced larger clutches than *P. phalangioides* (Fig. 1), similar numbers of spiderlings emerged from the eggsacs of each species (Table 1), which likely offsets the advantage of producing more eggs. Nevertheless, the variability in the number of spiderlings emerging from eggsacs was very high for both species. This variation was particularly remarkable for our observations of the matings of virgin spiders since they had been in the laboratory on a standard feeding schedule for at least one month prior to mating. In a study of *P. phalangioides*, Hoefler et al. (2010) found that males with prior experience had higher mating success than those who had never been exposed to female silk. However, we typically hold animals in the laboratory in individual containers so as to control for mating and prevent cannibalism. It may be that these protocols impact the mating system and the propensity for males to transfer, or females to accept, sperm, which might account for the high variability in offspring production we observed.

We uncovered a negative relationship between egg size and number for *P. phalangioides* yet no such relationship emerged for *P. manueli* (Fig. 1). Theoretically, such a trade-off between making more eggs vs. making larger eggs should occur when there are constraints on resources (Bernardo 1996; Roff & Fairbairn 2007). Presumably larger eggs translate to larger offspring which potentially increases their success (Walker et al. 2003; Warne & Charnov 2008). In addition, some of the cost of the smaller clutch sizes we observed for *P. phalangioides* is offset by the fact that individuals in our sample were more likely to produce additional eggsacs. Thus, these data alone would also cause us to predict that *P. phalangioides* would be more successful than *P. manueli*. On the other hand, the larger clutch size of *P. manueli* was not related to egg size. This result suggests that this species maximizes clutch size within the constraint on some minimum viable offspring size (Marshall & Gittleman 1994). Alternatively, it is possible that the excess eggs in the sacs of *P. manueli* are trophic eggs that support the success of the developing spiderlings and offset the fact that the eggs themselves are smaller in size. Trophic eggs have been reported for a variety of arthropod species, including some spiders (Perry & Roitberg 2006). In any event, these data suggest that there should be less variation in offspring performance for *P. manueli* than for *P. phalangioides*.

Our data failed to support the hypothesis that a higher reproductive potential aided *P. manueli* in displacing and excluding *P. phalangioides*. In fact, the higher reproductive rate and the larger egg size could give *P. phalangioides* an advantage over its competitor. Having a numbers advantage can be critical for both invading species and those native species trying to resist displacement (Blackburn et al. 2015). Thus, there must be some other aspect of the biology of *P. manueli* that is leading to its success in replacing *P. phalangioides*. Since virtually nothing is known regarding the basic biology and behavior of *P. manueli*, the information we report here may help frame subsequent studies that address their successful invasion, ecology, and evolutionary history.

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SHORT COMMUNICATION

Egg sacs of *Liocranoides* Keyserling, 1881 (Araneae: Zoropsidae) cave spiders

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Abstract. Little is known about reproduction in most cave spiders, including reproductive behaviors, seasonality, and fecundity. In the course of fieldwork in Tennessee caves, we observed aspects of reproduction in three populations of *Liocranoides* Keyserling, 1881 (Araneae: Zoropsidae) spiders. We observed egg sacs of *L. archeri* Platnick, 1999, as well as egg sacs and spiderlings of *L.* cf. *gertschi* Platnick, 1999. The spiders produced a spherical egg sac that hung from the cave ceiling by a single cord of silk. The egg sacs were covered by foreign material including sediment, rocks, and plant roots. Egg sacs were observed in June and July, and spiderlings were observed in July. Three egg sacs that were collected contained 26, 42, and 53 eggs. This is the first description of reproduction in *Liocranoides*.

Keywords: Subterranean, reproductive behavior, reproduction

Spiders are members of subterranean communities around the world. More than 1000 spider species representing ~50 families exhibit morphological adaptations to subterranean life and many more species are associated with caves to varying degrees (reviewed in Mammola & Isaia 2017). Most cave spiders are poorly known, and little is known about their reproduction, including seasonality, fecundity, and reproductive behaviors. Here we describe egg sacs and observations of spiderlings from *Liocranoides* Keyserling, 1881 (Araneae: Zoropsidae) spiders in caves in Tennessee. This is the first description of reproduction in this genus.

Liocranoides consists of five described species (World Spider Catalog 2018). *Liocranoides unicolor* Keyserling, 1881 was described first, based on specimens from Mammoth Cave, Kentucky. Platnick (1999) revised the genus and extended the range of *L. unicolor* into north-central Tennessee. He also described four additional species from the southern Appalachians: *L. temessseensis* Platnick, 1999 (from central and eastern Tennessee), *L. coylei* Platnick, 1999 (southwestern Virginia, western North Carolina, and eastern Tennessee), *L. archeri* Platnick, 1999 (south-central Tennessee and northeastern Alabama), and *L. gertschi* Platnick, 1999 (northern Alabama and northwestern Georgia). However, there are multiple undescribed *Liocranoides* species present throughout the Appalachian region and members of this genus may exist as a species complex similar to *Nesticus* Thorell, 1869 (Araneae: Nesticidae) cave spiders found in Appalachia (Milne unpublished data; Hedin 1997). All five described species have been collected in caves, and two (*L. unicolor* and *L. archeri*) are known only from caves (Platnick 1999).

During a visit to Keith Cave (Franklin County, Tennessee; Tennessee Cave Survey (TCS) #FR14) on 1 June 2017, we observed nine *L. archeri* individuals (Fig. 1A), as well as three egg sacs hanging from the ceiling of the upper chambers of the cave. These spherical egg sacs were ~1 cm in diameter and hung from the ceiling by a thick cord of silk ~2 cm in length. The egg sacs were covered by fragments of sediment, rock, and plant roots (Fig. 1B). Two egg sacs had mature female *L. archeri* in close association (within 10 cm). We collected one egg sac (Tennessee Wildlife Resources Agency permit #1605) and found 53 cleavage stage embryos inside. Although foreign materials were attached to the outside of the egg sac, no foreign material was observed inside the egg sac (Fig. 1C). During a second visit to Keith Cave on 20 June 2017, we observed one egg sac and what appeared to be the remnant of an egg sac still attached to the ceiling (Fig. 1D).

Liocranoides archeri is known from ~20 caves in south central Tennessee and adjacent northeast Alabama (Platnick 1999; Lewis 2005; Dixon & Zigler 2011; Wakefield & Zigler 2012). *Liocranoides archeri* has only been collected from caves and so has been considered a cave-obligate species (Niemiller & Zigler 2013). It is pale and largely uniformly colored, which may be an adaptation to cave life (Fig. 1A). It does not, however, exhibit other obvious morphological adaptations to cave life (Platnick 1999). The spiders *Nesticus barri* Gertsch, 1984 (Araneae: Nesticidae) (Lewis 2005) and *Meta ovalis* (Gertsch, 1933) (Araneae: Tetragnathidae) are also known from Keith Cave. Both have egg sacs that are quite distinct from those described here (e.g., Carver et al. 2016; egg sacs of *M. ovalis* are similar to those of the European *M. menardi* (Latreille, 1804), as described in Lepore et al. 2012).

We observed a similar egg sac in Shinbuster Crawl Cave (Rutherford County, Tennessee; TCS #RU88) on 14 July 2017. Although there was no spider in attendance, we collected immature *Liocranoides* from the cave and assume it was a *Liocranoides* egg sac. We collected and dissected the egg sac and found 26 eggs inside. The material inside the egg capsules was disorganized, suggesting these eggs failed to develop and were degenerating. Last, we observed two intact egg sacs in East Fork Cave (Dickson County, Tennessee; TCS #D127) on 29 July 2017. One egg sac was collected and contained 42 cleavage stage embryos. A third egg sac in the cave was apparently recently hatched, with approximately 30 spiderlings on the ceiling surrounding the remnant of the egg sac. The spiderlings were nearly transparent with bodies 1–2 mm in width. We collected immature and mature *L.* cf. *gertschi* from the cave. The genitalia of the mature spiders differed slightly from the description of *L. gertschi*. All collected spiders have been retained by one of the authors (MAM) for further systematic study.

There are similarities between the egg sacs of *Liocranoides* and some other zoropsid taxa. Most notably, *Titiotus gertschi* Platnick & Ubick, 2008 hangs spherical egg sacs by a cord of silk from the ceiling of caves in California (Platnick & Ubick 2008). *Tengella perfuga* Dahl, 1901, from Nicaraguan forests, covers its spherical egg sacs with pieces of substrate including bark, soil, and leaves (Mallis & Miller 2017). Some other zoropsid spiders, including members of *Griswoldia* Dippenaar-Schoeman & Jocqué, 1997 and *Anstrotengella* Raven, 2012, encrust the egg sac with dirt and debris, but not all do (e.g., *Zoropsis* Simon, 1878) (Griswold 1991; Thaler & Knoflach 1998; Raven 2012).

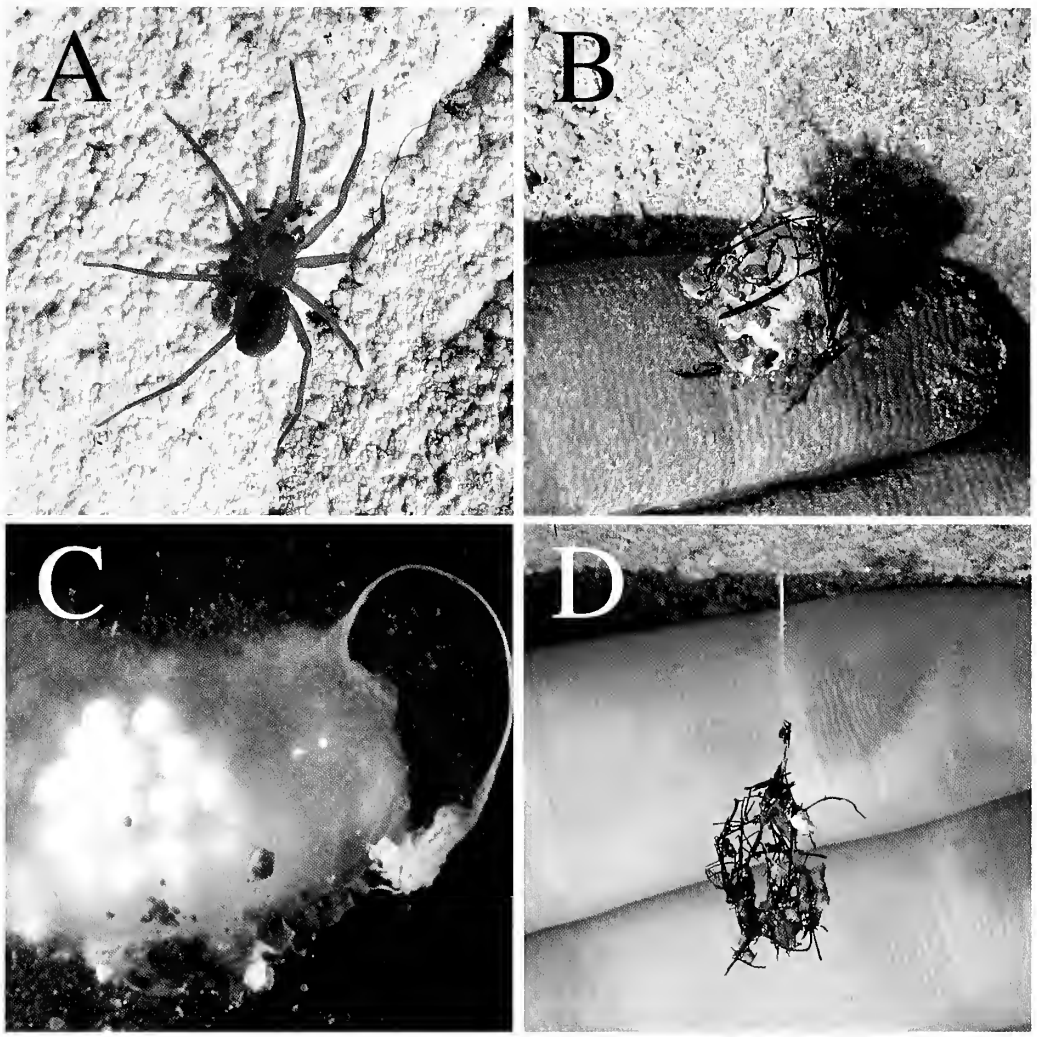

Figure 1.—*Liocranoides archeri* and egg sacs from Keith Cave (Franklin County, Tennessee). A. Mature female on cave wall (photo taken 20 June 2017). B. Egg sac hanging from the cave ceiling (photo taken 20 June 2017). C. Dissected egg sac. The cord of silk that attached the egg sac to the cave ceiling is at right. The egg sac contained 53 embryos and was collected on 1 June 2017. D. Remnant of an egg sac hanging from the cave ceiling (photo taken 20 June 2017).

The *Liocranoides* spiders and egg sacs we observed were in the twilight zone (the area to which some light penetrates) of these caves, rather than in dark zone cave habitats. The spiders were not found in webs and ran rapidly when disturbed. This suggests they are cursorial and hunt in the prevailing low light.

Over the past six years one of the authors (KSZ) participated in 241 visits to caves in Tennessee, Alabama and Georgia. The trips were distributed throughout the year, although fewer visits occurred in the winter (40/241). Across those visits these are the first observations of *Liocranoides* egg sacs, suggesting a brief or sporadic reproductive period for *Liocranoides*. Our anecdotal observations of *Liocranoides* egg sacs and spiderlings in June and July are consistent with observations of reproduction in *Nesticus* cave spiders. *Nesticus* cave spiders from the southern Appalachians exhibit reproductive seasonality with a peak in June and July (Carver et al. 2016). Further observations are required to determine if *Liocranoides* exhibit a similar pattern of reproductive seasonality.

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SHORT COMMUNICATION

Immolation of Museu Nacional, Rio de Janeiro – unforgettable fire and irreplaceable loss

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In 1807, the Portuguese royal government made a strategic retreat in advance of Napoleon I's invasion of Lisbon, and transferred the government to its thriving ex-colony of Brazil. Rio de Janeiro became the capital of the United Kingdom of Portugal, Brazil and the Algarves.

The Brazilian National Museum was established in Rio de Janeiro (MNRJ) in 1818, in the wake of great scientific and cultural improvement brought to Brazil by King John VI of Portugal (1767–1826). John VI's daughter-in-law, Archduchess Maria Leopoldina of Austria (1797–1826), was responsible for promoting visits by prominent European naturalists of the 19th century. Unfortunately, the collections made by these naturalists were taken to Europe. For example, the arachnological specimens of the Spix and von Martius expedition were taken to Munich (Horn & Kahle 1937) and destroyed by their use as teaching material (Tiefenbacher 1992).

The scientific enthusiasm of Emperor Pedro II of Brazil (1825–1891), who was born in Rio de Janeiro, promoted the early growth of the MNRJ collections, including an improbable stash of Ancient Egyptian steles and sarcophagi confiscated from smugglers and hundreds of artifacts of Pre-Columbian archaeology from the Emperor's personal collection. By then, MNRJ was already located in the Paço de São Cristóvão (Saint Christopher's Palace).

Growth of the zoological collections of MNRJ accelerated in the early 20th century when the arachnology collection was formed. Cândido de Mello-Leitão (Fig. 1a) was at first a collaborator and then a member of the museum staff. He was a pioneer and the most prominent arachnologist in South America; most of his personal collection was housed in the MNRJ.

From the mid-1990s onward, what is now called ArachnoLab (AraenoLab in Portuguese) entered a dramatic expansion phase. This included important output of scientific papers, training of taxonomists in Arachnida, Myriapoda and Onychophora, organization of expeditions around the world and multiplication of the holdings (Fig. 1b) of the arachnid/myriapod/velvet worm collections, including the purchase of the private collection of Helia Soares, containing many types of Opiliones.

The ArachnoLab welcomed several visitors each year, many interested in picking specimens of their own groups of interest from amidst our generous backlog (Fig. 1c). We also had a policy of sending abroad as many specimens as we could. Most reasonable requests from fellow arachnologists were met, and many scientists from the around world based a significant part of their research on the MNRJ specimens.

Dozens of successful students have been educated in the ArachnoLab (Fig. 1d), many of whom—such as A. Chagas (Universidade Federal do Mato Grosso - UFMT), A. Giupponi (Fundação Oswaldo Cruz - FIOCRUZ), A. Mendes (Universidade Estadual do Rio de Janeiro - UERJ), A. Pérez-González (Museo Argentino de Ciencias Naturales - MACN)—are presently employed in Arachnology/Myriapodology positions in educational and scientific institutions. Curator A.B. Kury's stimulating research on Opiliones functioned as a magnet for foreign students who received their M.Sc. and/or Ph.D. titles in Brazil. These hailed from Cuba (Abel Pérez-González), Colombia (Andrés García, Miguel Medrano) and Venezuela (Osvaldo Villarreal).

The Opiliones are the most intensively studied arachnid taxon at the MNRJ, and hundreds of important breakthroughs have been made though study of MNRJ material. However, in addition to Opiliones, meaningful discoveries based on material from our collection also include: (1) "the spider that was an Acari" (Krantz & Platnick 1995), (2) the amazing *Scolopendropsis duplicata* Chagas-Jr., Edgecombe & Minelli, 2008, a new centipede from Tocantins State, central Brazil, whose discovery led to a revised diagnosis of the order Scolopendromorpha (Chagas-Jr. et al. 2008), (3) *Troglorhopalurus translucidus* Lourenço, Baptista & Giupponi, 2004 (Buthidae), a bizarre, cave-dwelling, unpigmented scorpion (Lourenço, et al. 2004), (4) *Tmesiphantes hypogeus* Bertani, Bichuette & Pedroso, 2013 the first troglobitic tarantula from Brazil (Bertani et al. 2013), (5) material of the scorpion *Lychas scutilus* (C.L. Koch, 1845)(Buthidae), which allowed the designation of a neotype resolving a long-standing taxonomic problem (Lourenço 2017), (6) a new species of the amblypygid *Charinus* Simon, 1892 (Fig. 1c), endemic to the pluvial galleries of the museum palace itself, which became the symbol of the ArachnoLab. This species, which is in the process of description, may now be extinct, killed by the intense heat that radiated from the fire to the catacombs.

On September 2, 2018, just after the celebration of the 200th anniversary of the museum, the palace was entirely burned, causing the loss of all collections of Archaeology, Entomology, Ethnology, Malacology, Paleontology, and.... Arachnology (Fig. 1f). The scientific collections located in the ArachnoLab just before the cataclysmic fire comprised 190,000 specimens, of which ca. 2,000 were type specimens, including a fine Dipluridae collection, impressive collections of whip-spiders and scorpions (all with important types), and the crown jewel: the fantastic harvestmen collection (45,000 numbered specimens), equal in size to the spider collection and a particular specialty of MNRJ. This collection boasted a wealth of Colombian/Ecuadorian harvestmen and specimens from dozens of far-flung countries such as Indonesia and Tajikistan. The ArachnoLab also produced the OmniPaper project—a unique bibliographic online resource for taxonomy of Opiliones which is regularly visited by hundreds of students and researchers. The existence of OmniPaper is remarkable in itself, because it was produced without significant institutional support, and in a country where a bibliography is a rare commodity.

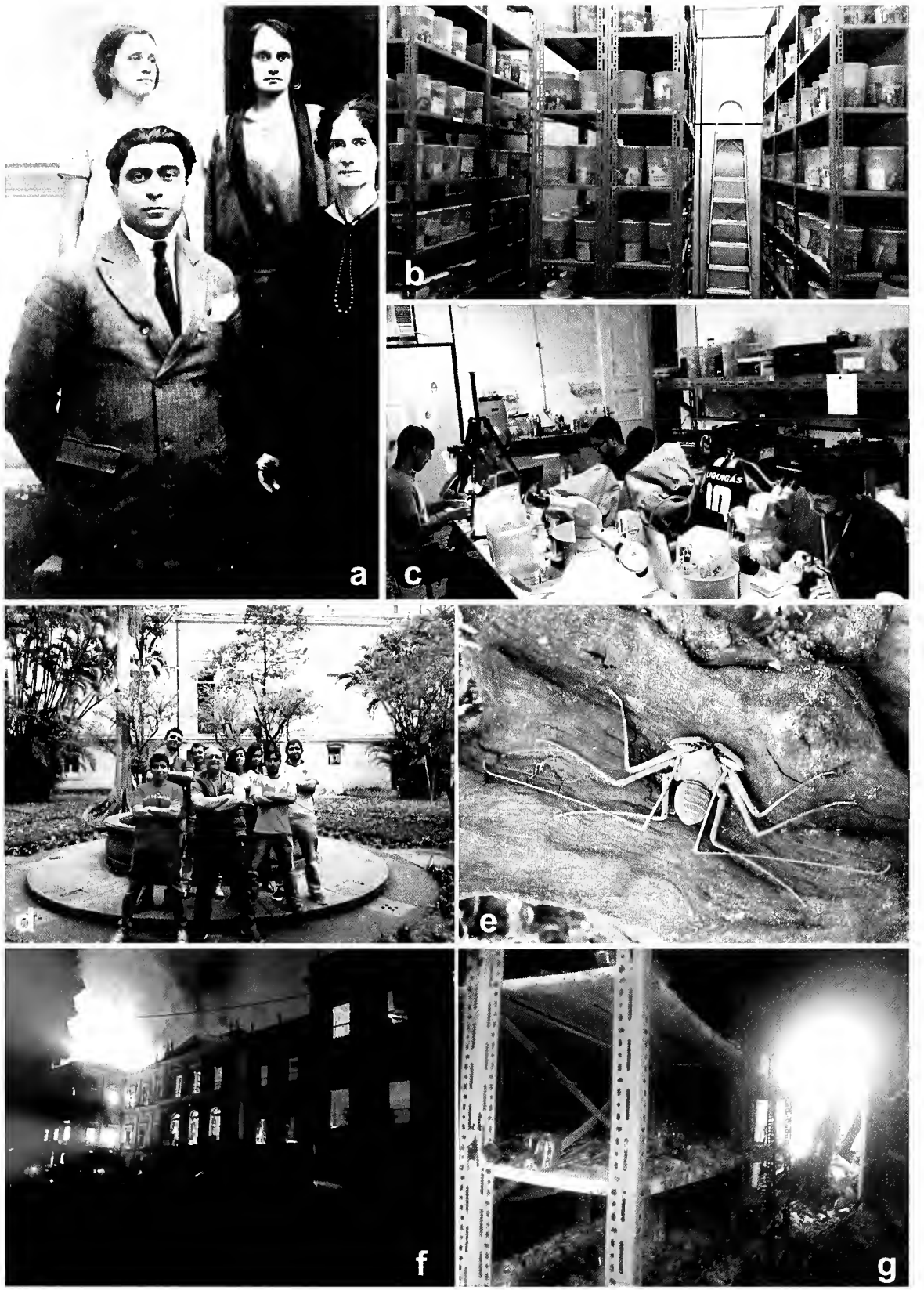

Figure 1.—a. Mello-Leitão in the late 1920s (from family album), b. Partial view of scientific collection of AraehnoLab, ca. 2016 (photo M. Medrano), c. Visit of researchers from Universidade de São Paulo, AraehnoLab (photo A.B. Kury), d. Team of the AraehnoLab in the central garden of the palace, 2016 (photo R. Gomes), e. Undescribed *Charinus* from the National Museum pluvial galleries (photo P.M. Costa), f. Moment of the explosion of the AraehnoLab (photo P.M. Costa), g. Aftermath of the fire, with the arachnological collection destroyed (photo A. Giupponi).

Figure 2.—Recovery efforts in the ArachnoLab; photo by Carla Barros.

All materials from the arachnology collection that were in the palace at the time of the fire were destroyed. However, all is not lost. Significant holdings were spared because they were on loan to other institutions, and this may include some of the material cited in this paper. Thanks to a serious backup routine (which unfortunately seems to have been an exception in the MNRJ), none of the electronic data of the arachno-collections and projects was lost in the cataclysm. Two type-specimen catalogues of the arachno-collection of MNRJ had already been produced: minor orders (Kury & Nogueira 1999) and a massive catalogue of spiders (Silva-Moreira et al. 2010). The culminating part, the catalogue of Opiliones types, was in preparation at the time of the fire.

In spite of our best efforts to create a center of arachnological research in the face of poor conditions for science in Brazil, the worst happened. When the 200-year old palace that lodged most of the MNRJ burst into flames, there was no water to quench the fire (Zamudio et al. 2018). A wealth of one fifth of a million specimens was lost to mankind (Fig. 1g), due to long-term neglect of the museum infrastructure.

Steady action should be taken to counteract the endemic neglect of education, science and culture in Brazil. Only when scientific research is treated with greater regard will our inheritance be preserved. Meanwhile, we have to rely only on the relentless personal efforts by Kury and colleagues to rebuild the ArachnoLab and a healthy collection (Fig. 2), which will again be open to all researchers of the world once the new legal barriers to the investigation of biodiversity (Bockmann et al. 2018) are cleared. The current way that people can contribute funds to help the rebuilding effort is shown on the website of ArachnoLab (online at http://www.museunacional.ufrj.br/mndi/Aracnologia/aracnol.htm).

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Citation of taxa in the text: Include the complete taxonomic citation (author, year) for each arachnid genus and/or species name when it first appears in the abstract and text proper. For example, *Araneus diadematus* Clerck, 1757. For Araneae, this information can be found online at www.wsc.nmbe.ch. Citations for scorpions can be found in the *Catalog of the Scorpions of the World (1758–1998)* by V. Fet, W.D. Sissom, G. Lowe & M.E. Braunwalder. Citations for the smaller arachnid orders (pseudoscorpions, solifuges, whip scorpions, whip spiders, schizomids, ricinuleids and palpigrades) can be found at museum.wa.gov.au/catalogues-beta/. Citations for some species of Opiliones can be found in the *Annotated Catalogue of the Laniatores of the New World (Arachnida,Opiliones)* by A.B. Kury.

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